Procedures for the Interpretation of GlobalFiler™ and Identifiler® Plus Data

1 Scope

These procedures apply to DNA personnel who verify and interpret nuclear DNA typing results obtained from the GlobalFiler™ and/or Identifiler® Plus PCR Amplification Kit using the GeneMapper® ID-X (GMIDX) DNA typing software for forensic comparison purposes and perform interpretation and statistical analysis using STRmix™.

2 Background

Upon completion of the technical aspects of nuclear DNA analysis, the results must be verified and interpreted by an Examiner. The verification of the accuracy of the results involves a review of peak designations and other information generated by the appropriate DNA typing software, as well as an evaluation of quality controls. Following this assessment, the Examiner makes comparisons among samples and draws conclusions that are captured for documentation and communication purposes within an FBI *Laboratory Report* (7-1, 7-1 LIMS, 7-273, or 7-273 LIMS).

The results are derived through application of the appropriate software during and after capillary electrophoresis (CE) of amplified DNA that is generated for each specimen using the GlobalFiler™ Amplification Kit with 28 cycles of PCR for all sample types. The number of contributors to the DNA typing results is determined, and based on a visual comparison of the DNA typing results, the Examiner may conclude that a person of interest (POI) is excluded as a possible contributor. DNA profiles from which a POI cannot be visually excluded as a possible contributor may be imported into the STRmix™ software.

STRmix™ calculates a likelihood ratio (LR) that reflects the probability of the DNA typing results under two opposing hypotheses: H_1, which includes the POI as a contributor to the evidence, and H_2, which does not include the POI as a possible contributor. These hypotheses typically align with the prosecution and defense positions, respectively, and as such are sometimes referred to as the prosecution hypothesis (H_p) and the defense hypothesis (H_d). The value of the LR leads to the conclusions that are captured within a written report.

The reinterpretation of legacy data from the Identifiler® Plus PCR Amplification Kit will follow these procedures and reporting language with supplemental information specific to the Identifiler® Plus data noted in Appendix E. For interpretation of legacy STR amplification kit data using a statistical approach other than STRmix™ (i.e., random match probability, combined probability of inclusion, or kinship analysis), refer to the procedures for Interpretation of Legacy DNA Data (i.e., DNA 230).

Familial comparisons of DNA results will be conducted using the appropriate interpretation protocol of the *DNA Procedures Manual* (i.e., DNA 227).

3 Equipment/Materials/Reagents

GeneMapper® ID-X Software (Applied Biosystems, version 1.6 or higher)

STRmix™ (NicheVision Forensics LLC, version 2.4 or higher)

4 Standards and Controls

Raw data for the electrophoretic runs of samples or controls displaying no typing results must be reviewed for the presence of a primer peak. If no primer peak is observed, the sample must be reinjected or reprepared to verify that amplicon was added to the CE plate.

4.1 Verification of GeneScan™ 600 LIZ v2.0 Internal Size Standard (GS-600v2)

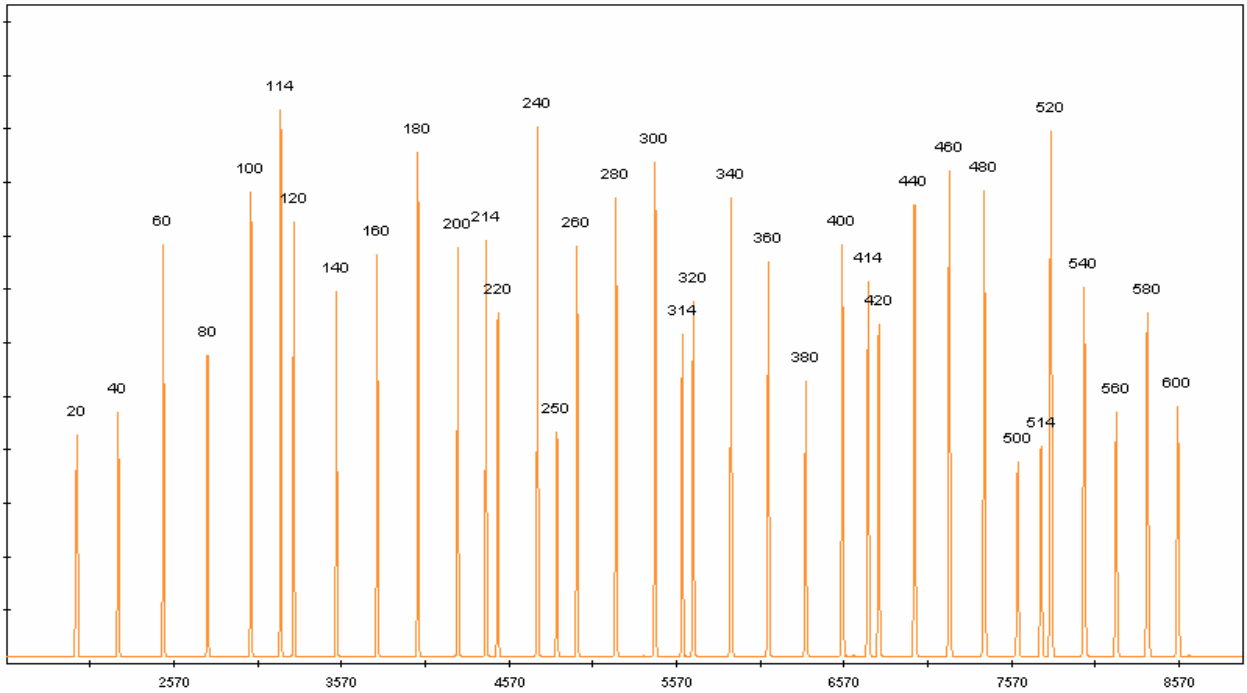

Figure 1 – GS-600v2 Internal Size Standard Peak Series

4.1.1 For GlobalFiler™ data, which is sized using the Local Southern Method, verify that the 60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440, and 460 base pair (bp) fragments of the GS-600v2 (Figure 1) are captured and have been assigned the correct size values for each sample, control, and allelic ladder. Fragments 60 through 460 must be captured and assigned the correct size values for a given injection of a sample to be interpreted. Note: Due to the temperature sensitivity of the 250 bp fragment's sequence-based conformation, this fragment is not used for sizing purposes.

4.1.2 If all of the GS-600v2 fragments for a given injection of a sample do not meet these specifications, a different injection of the sample that does display the correct size values for all of the GS-600v2 fragments must be used for interpretation of the entire DNA profile, which may require that the sample be reprocessed.

4.2 Verification of Allelic Ladders

Locus	Known Size Range (bp)[1]	Alleles Present in Ladder[2]	Color
D3S1358	96 - 142	9 - 20	Blue
vWA	156 - 209	11 - 24	Blue
D16S539	227 - 268	5, 8 – 15	Blue
CSF1PO	283 - 319	6 – 15	Blue
TPOX	338 - 379	5 – 15	Blue
Y indel	81 - 86	1, 2	Green
Amelogenin	98 - 105	X, Y	Green
D8S1179	114 - 171	5 - 19	Green
D21S11	183 - 240	24, 24.2, 25, 26, 27, 28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36, 37, 38	Green
D18S51	261 - 343	7, 9, 10, 10.2, 11, 12, 13, 13.2, 14, 14.2, 15 - 27	Green
DYS391	365 - 389	7 - 13	Green
D2S441	76 - 114	8 – 11, 11.3, 12 - 17	Yellow
D19S433	118 - 172	6 – 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18.2, 19.2	Yellow
TH01	179 - 218	4 – 9, 9.3, 10, 11, 13.3	Yellow
FGA	223 - 379	13 – 26, 26.2, 27 – 30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2	Yellow
D22S1045	88 - 121	8 – 19	Red
D5S818	138 - 183	7 – 18	Red
D13S317	199 - 243	5 – 16	Red
D7S820	262 - 299	6 – 15	Red
SE33	307 - 429	4.2, 6.3, 8, 9, 11 – 20, 20.2, 21, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 27.2, 28.2, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35, 35.2, 36, 37	Red
D10S1248	85 - 130	8 – 19	Purple
D1S1656	160 - 207	9 – 14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18.3, 19.3, 20.3	Purple
D12S391	216 - 269	14 – 19, 19.3, 20 – 27	Purple
D2S1338	281 - 350	11 – 28	Purple

Table 1 – GlobalFiler™ Allelic Ladder Specifications

[1] Sizes are based on Applied Biosystems' precision studies of the GlobalFiler™ Allelic Ladder published in the *GlobalFiler™ User Guide*. Sizes may vary due to electrophoretic effects.
[2] Unless otherwise indicated, ranges of alleles (e.g., 12 – 19) include only integers (e.g., 12, 13, 14, …, 19).

4.2.1 Any allelic ladder used for genotyping must: 1) exhibit the correct allele designations (see Table 1) and 2) yield the correct typing results when used to genotype the positive amplification control.

4.2.2 If any sample(s) requires reinjection, the appropriate ladder must be included in the reinjection set.

4.3 Positive Amplification Control (i.e., 007)

4.3.1 One positive control must be processed in parallel with each amplification set or batch of samples.

4.3.1.1 If any sample(s) requires repreparation, the positive control and the appropriate ladder must also be reprepared.

4.3.2 If a set of samples has multiple injections of the positive control, at least one injection must exhibit all the expected allelic peaks ≥ 150 relative fluorescence units (RFU), and must not exhibit any extraneous allelic peaks. A positive control with a non-allelic peak(s) (e.g., stutter, spike, pull-up) may be interpreted. See Table 2 for the expected positive control typing results obtained using GlobalFiler™.

Locus	007
D3S1358	15, 16
vWA	14, 16
D16S539	9, 10
CSF1PO	11, 12
TPOX	8
Y indel	2
Amelogenin	X, Y
D8S1179	12, 13
D21S11	28, 31
D18S51	12, 15
DYS391	11
D2S441	14, 15
D19S433	14, 15
TH01	7, 9.3
FGA	24, 26
D22S1045	11, 16
D5S818	11
D13S317	11
D7S820	7, 12
SE33	17, 25.2
D10S1248	12, 15
D1S1656	13, 16
D12S391	18, 19
D2S1338	20, 23

Table 2 – Expected DNA Typing Results of the Positive Control for STR Loci Analyzed Using the GlobalFiler™ Kit

4.3.2.1 If the positive control does not exhibit the expected results, one or more of the following actions must be taken:

a. The positive control may be regenotyped using an alternate ladder(s) that was run in parallel with the positive control. If this positive control then exhibits the expected results, any samples that are successfully genotyped using the alternate ladder may be interpreted.

b. If regenotyping of the positive control does not result in the expected results, the positive control may be reinjected using the same CE daughter plate together with a ladder. If the positive control then exhibits the expected genotype, all samples may be interpreted using the ladder(s) that produces the expected positive control genotype.

c. Alternatively, the positive control may be reprepared onto a new CE daughter plate together with a ladder and all samples that were injected in parallel. If the positive control displays the expected genotypes, all samples that were injected from the reprepared CE daughter plate may be interpreted.

d. If additional allelic peaks are present in the positive control (i.e., it is contaminated), then all samples amplified in parallel with this control should be assessed as described in section 5.2.

e. If after reinjection and/or repreparation, the results indicate that a DNA source other than the positive control was substituted for this control DNA, or if the positive control displays no results, then any samples amplified in parallel with this control may not be interpreted.

4.4 Negative Amplification Control

A negative control must be processed in parallel with each amplification set or batch of samples. If the negative control does not result in the expected results, one or more of the following actions must be taken:

a. If at least one extraction control or negative sample on the original plate is clean, the negative control may be reprepared onto a new CE daughter plate together with a ladder and the positive control. If the negative control displays the expected results, all samples may be interpreted.

b. Alternatively, if there are no clean extraction controls or negative samples on the original plate, the entire plate may be reprepared onto a new CE daughter plate. If the negative control displays the expected results, all reprepared samples may be interpreted.

c. If the negative control still does not display the expected results, see sections 5.1.1.1 (Identification of Peaks of Non-Genetic Origin) or 5.2 (Contamination) for guidance on how to treat unresolved peaks observed in the negative control.

4.5 Extraction Control (i.e., Reagent Blank)

4.5.1 At least one extraction control must be processed in parallel with each batch of samples subjected to a specific extraction process.

4.5.2 If a sample needs to be reamplified or reinjected, and the extraction control processed in parallel with this sample does not have any typing results, then the extraction control does not need to be reprocessed.

See sections 5.1.1.1 (Identification of Peaks of Non-Genetic Origin) or 5.2 (Contamination) for guidance on how to treat peaks observed in extraction controls.

5 Procedures

5.1 DNA Profile Determination

5.1.1 Computer Assisted Allele Designations

The GMIDX software, using the analysis method settings for GlobalFiler™ data represented in Appendix A, analyzes the data generated by the CE instruments and generates electropherogram data to be evaluated and interpreted. The analysis method settings for Identifiler® Plus data are contained in the procedure for interpretation of results from the Identifiler® Plus amplification kit (i.e., DNA 229) and/or the procedures for interpretation of legacy DNA data (i.e., DNA 230). A pink box surrounding a data point label indicates that the software has identified a data point as an artifact. The GMIDX software uses the terms "spike" and "OMR" (Outside Marker Range) to represent a variety of DNA artifacts. Peak labels may be edited according to this SOP. Peaks interpreted as non-allelic may be deleted within GMIDX and will appear on the electropherogram with a single strikeout.

5.1.1.1 Identification of Peaks of Non-Genetic Origin

Before the STR typing results from a sample can be used for comparison purposes, it is necessary to identify any non-genetic peaks that do not represent human, allelic STRs. These non-genetic peaks may be undesired PCR products (e.g., stutter, -A, and non-specific product), analytical artifacts (e.g., spikes and raised baseline), instrumental limitations (e.g., matrix failure), or be introduced into the process (e.g., disassociated primer dye and non-specific peaks).[3] Additionally, data may have distinctive characteristics consistent with non-human DNA. The various types of peaks are described below.

Reproducible peaks (e.g., stutter, -A, disassociated dye, matrix failure, non-specific product) may be interpreted.[4] Non-reproducible peaks (e.g., spikes and raised baseline) must be evaluated as specified.

[3] The GMIDX software may apply a filter that removes labels from peaks at any locus that meet the FBI-defined sizing and relative peak height criteria for stutter, and 10% for minus-A.
[4] For purposes of interpreting DNA typing results, a peak need only be identified as being of non-allelic origin.

5.1.1.1.1 Stutter

The kit-specific stutter percentage guidelines provided in Table 3 are estimates of the maximum expected stutter values at each locus in the GlobalFiler™ amplification kit. These values are expressed as a percentage relative to the source allelic peak height (i.e., % Stutter). Stutter may occur at other locations, such as minus-two repeat units, or be excessively high for a sample. If such atypical stutter peaks are due to excessive amounts of template DNA, the sample may be reamplified with less template DNA or reinjected for less time. These values are the stutter percentages incorporated into GMIDX when the stutter filter is used. STRmix™ models stutter using a different method.

Locus	- 1 Repeat Unit	-2 Repeat Units	- 2bp	+1 Repeat Unit
D3S1358	11	1	N/A	2
vWA	11	1	N/A	3
D16S539	10	2	N/A	3
CSF1PO	10	N/A	N/A	6
TPOX	6	N/A	N/A	N/A
D8S1179	10	2	N/A	3
D21S11	11	3	N/A	3
D18S51	13	2	N/A	5
DYS391	8	2	N/A	5
D2S441	8	1	N/A	2
D19S433	10	2	N/A	3
TH01	4	N/A	N/A	N/A
FGA	12	2	N/A	4
D22S1045	18	2	N/A	8
D5S818	10	2	N/A	2
D13S317	10	1	N/A	3
D7S820	9	2	N/A	4
SE33	14	2	5	5
D10S1248	12	2	N/A	4
D1S1656	12	3	3	3
D12S391	14	2	N/A	3
D2S1338	12	2	N/A	4

Table 3 – Maximum Expected Stutter Percentage Guidelines for STR Loci Analyzed Using the GlobalFiler™ Kit

5.1.1.1.2 Minus-A (–A)

It is expected that –A generally occurs at less than 15% for peaks that are not off-scale. The interpretation of –A peaks is based on the following:

a. (1) whether the DNA results are derived from a single contributor or whether the N-1 peak may represent an allele from an additional contributor(s) to the sample (this determination will entail multiple loci), (2) the size and relative peak height of the N-1 peak, (3) whether an additional allele(s) at the same locus exhibits an N-1 peak, and (4)

whether there is any indication of excessive DNA template and/or amplification inhibition.

b. If –A peaks are extensive and/or interfere with interpretation, the sample may be reinjected or reamplified.

5.1.1.1.3 Pull-Up Resulting from Spectral Failure

Pull-up is assessed using the following criteria.

a. The resultant artifactual peak(s) (usually less than 10% of the source peak) typically sizes within ± 0.25 bp (± approximately two scan units) of its source peak. The sizing of a pull-up peak may be distorted if it occurs sufficiently close to another peak in the same color (i.e., the two peaks overlap with respect to the X-axis). Such peaks may display a size difference of greater than ± 0.25 bp relative to its source peak.

b. Samples exhibiting extensive spectral failure or spectral failure that interferes with interpretation may be reinjected for less time or reamplified with less template DNA.

5.1.1.1.4 Spikes

a. Spikes are generally detected in two or more colors and typically size within ± 0.15 bp of each other. The morphology of spikes can vary greatly from that of an allelic peak. Depending on whether a spike sizes within a bin it may be labeled as either an allele or an off-ladder (OL) allele.

b. If a spike occurs at or sufficiently close to an internal size standard peak or a ladder peak such that sizing and/or allelic designation is affected, these samples must be reinjected.

c. Because a spike(s) within a sample or control (i.e., extraction control and/or negative amplification control) from which no typing results are obtained may mask an allelic peak(s), any such sample or control that displays a spike(s) within the expected size range of a locus that yields relatively small allelic fragments (generally 200 bp in size or less) must be reinjected. Spikes that occur within the size ranges of genetic loci that yield larger allelic fragments may be reinjected.

5.1.1.1.5 Dissociated Primer Dye

The interpretation of dye peaks is based on the following:

a. The peak's morphology (e.g., dye peaks are generally much broader than peaks of DNA origin).

b. The presence of peaks of similar size and morphology in other samples and/or controls amplified together with the sample or control being evaluated.

c. Whether the observed peak occurs within a size range previously associated with a dye peak from a previous lot of amplification kit.

5.1.1.1.6 Non-Specific Peaks

Non-specific peaks are defined as peaks of unknown origin. Some of those known to be associated with specific commercial products are noted below. Additional artifacts are described in the product user guides or technical notes distributed by the manufacturer.[5]

a. A reproducible artifact intrinsic to the GlobalFiler™ Kit may be detected at the TH01 locus, typically sizing approximately 12 nucleotides smaller than a TH01 allele. The kit manufacturer specifies that these artifacts range from 0.4% to 0.9% of the TH01 allele peak heights.

b. A reproducible artifact intrinsic to the GlobalFiler™ Kit may be detected at the TPOX locus, typically sizing approximately 24 nucleotides smaller than a TPOX allele.

c. A reproducible artifact intrinsic to the GlobalFiler™ Kit may be detected between the amelogenin and Y indel loci, typically sizing at ~94.5bp, at 0.3% to 0.7% of the X allele peak and not falling within an allelic bin. The peak height is directly proportional to the peak height of the X allele peak.

5.1.1.1.7 Raised Sample Baseline

A sample(s) that displays excessive raised baseline (i.e., an elevated horizontal axis at, or between, two or more loci that results in stretches of non-specific, low amplitude data) should be reinjected using the standard injection time or for reduced time(s). If such subsequent injections do not result in on-scale data, the sample(s) may be reamplified with less DNA template and/or reextracted.

5.1.1.1.8 Non-Human Peaks

The amplification of non-human DNA may exhibit characteristics such as:

a. A peak at ~98 bp (before amelogenin) and/or randomly at other loci.
b. A peak at ~207 bp detected at the D21 locus.
c. A peak at ~204 bp detected at the vWA locus.
d. Alleles absent at the majority of the locations but not consistent with a degraded profile or low level human DNA sample.

Non-human DNA typing results should not be used for comparisons.

5.1.1.2 Excessive DNA Template and Off-Scale Samples

An excessive amount of template DNA may result in the appearance of off-scale peak(s), which exceed the linear dynamic range of the genetic analyzer detector. These samples may also exhibit

[5] See, for example: ThermoFisher Scientific. Technical Note: Artifacts Identified Post-Developmental Validation: GloabalFiler™ PCR Amplification Kit. 2019. Available at www.thermofisher.com.

raised baseline, pull-up peaks, -A, atypical stutter, and/or non-specific peaks in one or more colors. If the artifacts are excessive, these samples may be reinjected for reduced time(s), reamplified with less DNA template, and/or reextracted. Off-scale peaks may be displayed by the major contributor to a mixture of DNA when a large difference exists between the major and minor contributions to the sample. In such cases, allelic peaks from the minor contributor may be interpreted in the presence of off-scale major contributor peaks provided that no excessive raised baseline and/or excessive non-specific peaks are present. If necessary, a locus exhibiting excessive non-specific peaks may be deemed inconclusive for matching/statistical purposes.

5.1.1.3 Off-Ladder (OL) Alleles

If an allele fails to size within a defined allele category (e.g., a bin or a virtual bin), it must be assessed using the following criteria.

5.1.1.3.1 Any sample containing an OL allele may be reinjected.

5.1.1.3.2 An OL allele may be a microvariant that sizes between two ladder alleles. For example, if an OL allele occurs between the 12 and 13 ladder alleles and is approximately 1 bp larger than the 12 allele, it is designated as 12.1; 2 bp larger is designated 12.2; and so on.

5.1.1.3.3 If an OL allele does not fall within the size range of any locus-specific ladder, which includes the flanking virtual bins, it must be associated with one of the two loci between which it falls.

a. For single-source samples, if the OL allele is flanked by a locus with two peaks and a locus with a single peak, the OL allele is assigned to the latter locus.
b. If an OL allele falls between two loci that both display either a single allele or two or more alleles, the OL allele may be assigned to the locus closest in size to the OL allele. Peak height evaluation may also aid in the assignment of the OL allele to a locus.[6]
c. If determination of the locus assignment is not possible, both loci that flank the OL allele must be deemed inconclusive for matching/statistical purposes.
d. If the OL allele is smaller in size than the smallest respective virtual bin, or larger in size than the largest respective virtual bin, the number of repeats in the allele should be estimated for use in STRmix™ analysis.
e. When loci are closely spaced on the x-axis of an electropherogram, an above or below OL allele may be observed within the size range of a flanking locus. Peak heights, peak height ratios, the number of alleles in the flanking loci, and the size of the OL allele (e.g., whether it is approximately an integer value above or below the flanking ladder allele(s)) may be used to determine the appropriate locus designation for the OL allele.

5.2 Contamination Assessment

Although appropriate quality assurance practices are stringently applied and enforced, it is not unexpected that low-levels of adventitious DNA may be detected due to the highly sensitive nature of the amplification process. Adventitious DNA may be attributable to a specific source (e.g., laboratory

[6] To facilitate the interpretation of OL alleles, the Examiner may consult a listing of such alleles recorded at http://www.cstl nist.gov/div831/strbase/var_tab htm.

personnel) or be from an unknown source. Further, contamination may occur in either a random or systemic manner. Instances of contamination will be evaluated on an individual basis according to the following guidelines.

5.2.1 General Contamination Guidelines

5.2.1.1 If contamination occurs in a sample or control, the affected extraction batch and amplification batch should be assessed for random or systemic contamination. It is noted that systemic contamination may be limited to the extraction batch, leaving the remaining samples in the amplification batch unaffected. The determination of contamination as random or systemic may be complex; the Examiner should consult the Technical Leader (TL) for additional guidance as necessary.

5.2.1.2 If a control is affected by contamination, but the results from the sample indicate that only the expected contributor(s) was present or no results were obtained, the sample does not have to be reprocessed.

5.2.1.3 Other samples should be reprocessed as described:

- If the contamination is characterized as systemic, any sample processed in parallel with the systemic contaminant should be reprocessed if possible.
- If the contamination is characterized as random and occurs in a control, any sample processed in parallel with the affected control should be reprocessed if possible.
- If the contamination is characterized as random and occurs in a sample, the sample should be reprocessed if possible. It is not necessary to reprocess other sample(s) processed in the same batch.

5.2.1.4 If reprocessing is not conducted or possible, the resulting data may be interpreted in a conservative and cautious manner following the procedures described below.

5.2.1.5 Refer to the report wording section 5.5.7 for further direction on how to denote the contamination in the report.

5.2.2 Evidence Handler Contaminant

5.2.2.1 If the contamination event is consistent with having arisen from an individual who handled the sample at any stage of the process, and if reprocessing the sample is not possible or successful, it is possible to condition on the source of the contaminant. Conditioning on the source of the contaminant requires that the typing results support the presence of the contributor in the sample. The results may be used for interpretive purposes and for submission to the Combined DNA Indexing System (CODIS) with TL approval.

5.2.3 Random Contamination

5.2.3.1 If the contamination event is determined to be random, the samples processed in parallel may be used for interpretive purposes and may be submitted for inclusion in CODIS.

5.2.3.2 If a sample with random contamination is the basis for the conclusions in a report (i.e., reextraction was not possible or not successful), the occurrence of contamination must be noted in the report.

5.2.3.2.1 If a single-source or major contributor in an evidentiary sample is determined to be a random contaminant (with the exception of an evidence handler contaminant, see 5.2.2), the results for the single-source or major contributor are not suitable for interpretive purposes and may not be submitted for inclusion in CODIS. Results for a minor contributor(s) may only be used for exclusionary purposes and may not be submitted for inclusion in CODIS.

5.2.3.2.2 If a minor contributor to a mixture in an evidentiary sample is determined to be a random contaminant (with the exception of an evidence handler contaminant, see 5.2.2), the results from that sample for a major contributor may be used only for exclusionary purposes and may not be submitted for inclusion in CODIS.

5.2.4 Systemic Contamination

5.2.4.1 If the contamination event is determined to be systemic (with the exception of an evidence handler contaminant, see 5.2.2), the data for all of the samples processed in parallel may not be used for interpretive purposes and may not be submitted for inclusion in CODIS as this demonstrates a failure in the analytical process. The occurrence of systemic contamination must be noted in the report.

5.2.4.2 With TL approval, exculpatory results (e.g., ability to exclude the subject from the male profile in a sperm fraction) should be reported. The contamination and the limitations of future comparisons must be noted in the report.

5.3 Application of Peak Height Thresholds to Allelic Peaks

5.3.1 The analytical threshold (AT) is 150 RFU; however, it can be lowered to 50 RFU for bones, tissues, hair, and/or teeth, as long as these samples present as single source. When a sample is analyzed with an AT of 50 RFU, the negative amplification control and the reagent blanks associated with the sample must also be analyzed using an AT of 50 RFU. The positive amplification control and ladders will be analyzed using an AT of 150 RFU.

5.3.2 The stochastic threshold (ST) is 725 RFU.

5.3.3 Because STRmix™ incorporates an empirically determined probability of dropout, no ST is used in the interpretation of samples subjected to direct comparisons using STRmix™. However, the ST and potential for allele dropout must be considered during visual comparisons that are the basis for a direct exclusion without the use of STRmix™.

5.3.4 The ST is applicable to typing results from questioned specimens that are used for familial comparisons and known specimens. For samples known or expected to be of single source origin (e.g., reference samples, alternate reference samples, bones, tissue) that display results consistent with having arisen from a single individual, the ST is applied to only those loci at which stochastic loss of information is possible (i.e., loci that display a single allelic peak < ST).

5.4 Interpretation of DNA Typing Results

To the extent possible, DNA typing results from evidentiary samples will be interpreted before the comparison with any known samples, other than those of assumed contributors.

When there are multiple amplifications and/or injections for a given sample extract, generally the one that provides the most information will be used for reporting. However, sample saturation or loss of resolution that interferes with interpretation may require that an alternative amplification/injection is used.

5.4.1 Peak Height Ratio

Peak height ratios (PHR) can be used to associate two alleles to a common source and to establish the presence of a DNA mixture.

5.4.1.1 Peak height ratios are calculated by dividing the peak height of the allele with the lower RFU value by the peak height of the allele with the higher RFU value, and then multiplying this value by 100 to express the PHR as a percentage.

5.4.1.2 The values provided in Table 4 are estimates for the minimum expected PHR percentages for the GlobalFiler™ Amplification Kit and are based on the average PHRs observed in the validation studies. The PHR guidelines are only applicable to allelic peaks that meet or exceed the ST.

Peak Height	All GlobalFiler™ STR Loci
725 – 1999 RFU	50%
2000 – 4999 RFU	60%
5000 RFU and above	70%

Table 4 – Minimum Expected Heterozygous Peak Height Ratio Guidelines

5.4.1.3 Because reference samples and human remains (e.g., bones, teeth, tissue) are attributable to a single individual, PHR assessments are generally not used in their interpretation. Should a mixture of DNA be obtained from such samples, the major contributor type can be assessed using the peak height ratios described in Table 4. A major contributor profile may only be used for an alternate reference sample if no other samples are available. Mixtures in human remains samples will not be submitted to CODIS unless approved by the TL.

5.4.2 Determination of the Number of Contributors to DNA Typing Results

5.4.2.1 The determination of the number of contributors to DNA typing results begins with counting the allelic peaks; therefore, peaks that exceed the expected stutter percentages must be evaluated considering:

- A peak significantly above the stutter percentage is more likely to be allelic.
- A peak at a small (<200 bp) locus where possible minor contributor types are expected has more potential to be allelic.

- A peak in an additive stutter position, which exceeds the negative stutter percentage but not the combined positive and negative stutter percentages, may be considered stutter.
- Other apparent peaks below the AT suggest that the peak is potentially allelic.
- If the sample is a reference sample and expected to be single source, then these peaks can confidently be called stutter if there is no other evidence of contamination.

5.4.2.1.1 For apparent single source samples, a peak in a stutter position that exceeds the expected stutter percentage may be interpreted as a stutter peak for purposes of determining the number of contributors to the sample. Generally, this interpretation is limited to a single instance unless the peaks are in the additive stutter position, at a large (>200 bp) locus, and there are no other indications of a mixture (e.g., peak height imbalance, apparent peaks <AT).

5.4.2.1.2 For mixed samples, peaks that exceed the expected stutter percentage are generally considered allelic for purposes of determining the number of contributors to the sample.

5.4.2.2 Using the locus with the largest number of alleles, divide the number of alleles by two, and round up to the nearest integer. This integer is the initial estimate of the number of contributors to the sample.

5.4.2.3 Again using the locus with the largest number of alleles, assess the ratio of contributors. Evaluate peak height imbalance and account for allele sharing to determine if the number of contributors should be increased. PHR assessments should generally be within empirically determined values for single source samples.[7]

5.4.2.4 Apply the general pattern of number of contributors and mixture ratio across the profile to determine if other loci are consistent with this pattern or if the number of contributors should be increased or decreased by one. Loci with more alleles will be the most informative for this assessment. Additionally, apparent peaks <AT may also be considered, especially for low level samples.

5.4.2.5 Samples in which three allelic peaks[8] are observed at a single locus, without any other indications of a mixture, may be concluded to be single source.

5.4.2.6 STRmix™ input files will include peaks in the one repeat unit forward or reverse stutter position, while peaks that have been interpreted as stutter at more than one repeat unit or at the -2 bp stutter position will remain filtered by GMIDX.

[7] Peak height imbalances may be seen in the results from a single individual due to elevated stutter, primer binding site variants that result in attenuated amplification of one allele of a heterozygous pair, or tri-allele patterns in which two copies of an allele are present within the genotype (e.g., a type 11,12,12).
[8] Observed tri-allele patterns are recorded at http://cstl.nist.gov/biotech/strbase/tri_tab.htm.

5.4.3 Sex Determination

Three loci in the GlobalFiler™ kit: Y indel, amelogenin, and DYS391, are used to determine whether male and/or female DNA is detected in a sample. Generally, male DNA will exhibit characteristic alleles at all three loci; however, an allele at only one or two Y-chromosome loci indicates the presence of male DNA. Missing alleles may be due to low quantity, degradation, or primer binding site mutations. The presence of male DNA in a mixed sample may limit the ability to determine if female DNA is also present in the sample.

5.4.3.1 Only female DNA is detected in a single source or mixed sample that exhibits both:

a) an X peak ≥ST at amelogenin in the absence of a Y peak and
b) no alleles at Y indel or DYS391

5.4.3.2 No conclusion regarding sex determination can be drawn if the sample exhibits both:

a) at amelogenin, an X peak <ST in the absence of a Y peak and
b) no alleles at Y indel or DYS391

5.4.3.3 Male DNA is detected in a sample that exhibits any of the following:

a) an allele at Y indel
b) a Y peak at amelogenin (with or without an X peak) and/or
c) an allele at DYS391

The sample should be reported as single source male, a mixture of male and female, or a mixture containing male DNA based on the following criteria:

5.4.3.3.1 If the sample is single source, it should be reported as male if any male characteristic alleles are detected, regardless of peak height.

5.4.3.3.2 If the sample is mixed, it should be reported as a mixture containing male DNA if either:

a) both the X and Y peaks are ≥ST and the Y/X PHR is ≥50% or
b) both the X and Y peaks are <ST

5.4.3.3.3 If the sample is mixed, it should be reported as a mixture containing male and female DNA if the Y/X PHR is <50% and either:

a) the X peak is ≥1450 (i.e., twice the ST), regardless of the peak height of the Y, or
b) both the X and Y peaks are ≥ST

5.4.3.3.4 If the sample is mixed, it may be interpreted as a mixture containing male DNA or containing male and female DNA when both:

a) the X peak is <1450 (i.e., twice the ST) and
b) the Y peak is <ST

The FE should consider Y/X PHR, the proportionate heights of the Y indel peak and/or the DYS391 peak, and the profile as a whole to make this determination.

5.4.3.4 For any sample with no alleles at amelogenin, Y indel, and DYS391, no sex typing results were obtained.

5.4.4 Exclusions Based on Visual Comparison of DNA Typing Results

Some exclusions may be declared upon visual comparison of typing results, as described below. No STRmix™ analysis is performed for exclusionary conclusions determined from visual comparisons.

5.4.4.1 Exclusions from Single-Source Profiles

An exclusion is declared when one or more loci in a single-source evidentiary profile is inconsistent with that of a known individual, considering dropout as applicable.

5.4.4.2 Exclusions from Mixed DNA Typing Results

5.4.4.2.1 To declare an exclusion upon visual comparison, the Examiner should consider the number of contributors, the number and height of alleles detected per locus, the height of stutter peaks, the potential for allele sharing among contributors, and the potential for allele dropout. Generally, the greater the complexity of the typing results (e.g., 3 or more contributors, few alleles detected per locus relative to the number of contributors, trace contributor(s), substantial peak imbalance), the greater the potential that the results should be interpreted using STRmix™.

5.4.4.2.2 Based on visual comparison, the Examiner may declare an exclusion to a mixed evidentiary profile if the known profile is not consistent with the potential genotype(s) in the mixture.

5.4.5 DNA Typing Results Subjected to STRmix™ Interpretation

5.4.5.1 STRmix™ analysis may be conducted on evidentiary typing results amplified using the GlobalFiler™ Kit with 28 cycles and separated on the Applied Biosystems 3500xL. Reference profiles are not constrained by these requirements for STRmix™ analysis.

5.4.5.2 STRmix™ is used for interpretation and statistical assessment of typing results from which a POI is not excluded based on visual comparisons. STRmix™ may also be used for mixture deconvolution in the absence of DNA typing results for a known individual.

5.4.5.3 Typing results assumed to originate from 1 to 4 contributors may be interpreted using STRmix™. Mixtures of DNA from 5 or more contributors will not be interpreted using STRmix™, and will be reported as unsuitable for comparisons.

5.4.5.4 A given sample may be interpreted in STRmix™ more than once if alternate hypotheses and assumptions relevant in the context of the case are being assessed.

5.4.5.5 Replicate amplifications of the same extract, with the same or different DNA template quantities, may be interpreted concurrently in the same STRmix™ analysis.

5.4.5.5.1 If a different extract is used for reamplification, the typing results cannot be analyzed as replicates in STRmix™, with the exception of bones, tissue, hair, and teeth with results that present as single source.

5.4.6 STRmix™ Settings

5.4.6.1 STRmix™ software settings, including FBI specific parameters from internal validation studies, are represented in Appendix B and Appendix F.

5.4.6.2 The value for Markov chain Monte Carlo (MCMC) accepts is set to 100,000 for burn-in and 500,000 total, and will not be changed without approval of the TL.

5.4.6.3 An F_{ST} setting of 0.01 is used for the African American, Caucasian, Southeastern Hispanic, Southwestern Hispanic, Chamorro, Filipino, and Trinidadian populations. For Native Americans populations (i.e., Apache, Minnesota Native American, and Navajo), an F_{ST} of 0.03 is used.

5.4.7 Propositions for Calculating the Likelihood Ratio (*LR*)

5.4.7.1 STRmix™ establishes two propositions (also referred to as hypotheses) based on user input to calculate the LR.

- The first proposition, H_1, generally includes the POI and, for mixed specimens, unrelated unknown (U) individuals. The total count of individuals included in the proposition is equal to the number of contributors in the sample.

  Example: For a three-person mixture, H_1 consists of the POI and two unrelated unknown individuals.

- The second proposition, H_2, generally consists of unrelated unknown individuals, equaling in total the number of contributors to the sample.

  Example: For a three-person mixture, H_2 consists of three unrelated unknown individuals.

5.4.7.2 Conditional propositions are used for mixed typing results when the presence of an individual's DNA in the sample can be reasonably expected, and the typing results support the presence of the contributor in the sample. In an analysis conditioned on a known individual, H_1 and H_2 include the same assumed contributor.

Example: for a three-person mixture:
H_1 = Assumed contributor + POI + U
H_2 = Assumed contributor + U + U

The assumed contributor(s) must be the first reference sample added to STRmix™. The assumed contributor(s) must be designated in H_2 using the "Change Hd" function in STRmix™. When an assumed contributor(s) is designated in H_2, STRmix™ reduces the number of unrelated unknown individuals as appropriate.

5.4.7.3 For cases with multiple POIs, each POI reference profile is analyzed individually in STRmix™ and reported separately. A request or scenario where it may be informative to report the LR of combined POIs must be approved by the TL.

Example: for a two-person mixture with POI_1 and POI_2:
H_1 = POI_1 + U and H_1 = POI_2 + U
H_2 = U + U H_2 = U + U

5.4.7.3.1 If multiple POIs yield inclusionary LRs (>1), the results should be assessed to ensure that the included POIs could be present in the mixture together. This assessment will done either visually (e.g., two POIs together do not account for all alleles in a two person mixture) or by running STRmix™.

5.4.7.3.2 If included POIs are interpreted to fit together in the mixture, they must be analyzed in STRmix™ together in a single analysis.

Example: for a three-person mixture with inclusionary POI_1 and POI_2:
H_1 = POI_1 + POI_2 + U
H_2 = U + U + U

5.4.7.3.3 If STRmix™ analysis of the combined POIs indicates that they can be present together in the mixture, the LRs run individually should be reported for each POI, and the combined LR should be maintained in the casefile.

5.4.7.3.4 If visual comparison (e.g., a limited single source profile that provides inclusionary LRs for 2 POIs) or STRmix™ analysis of the combined POIs indicates that they cannot be present together under the assumptions of the analysis, the profile and diagnostics may be reassessed to determine whether the analysis should be repeated under the same or different conditions (e.g., increasing the assumed number of contributors). If no change to the analysis is supported by the profile or diagnostics, the report should indicate this circumstance directly following the H_1 supporting LR table. For example:

Under the assumption that the DNA from item 1 originated from two individuals, it is not possible for both WHITE and JONES to be contributors together.

5.4.7.4 Evidentiary samples will be compared to all informative POIs. If there is no expectation that DNA from a particular POI would be present on an evidentiary sample, then no comparison to that POI is required for that sample.

5.4.8 STRmix™ Analysis

5.4.8.1 Information on software usage can be found in the STRmix™ User's Manual.

5.4.8.2 Tri-allelic loci in an evidentiary sample should be excluded from STRmix™ analysis by using the "Ignore locus" function. In a reference profile, alleles in tri-allelic loci should be deleted from the profile prior to import into STRmix™.

5.4.8.3 The Examiner will evaluate the STRmix™ results, including the diagnostics and weights of various genotypes relative to the DNA profile analyzed. In general, each STRmix™ analysis, consisting of the same evidence and reference profiles, propositions, and assumptions, will be run once and the results reported, except when an analysis has produced a result that requires further investigation and reanalysis. Examples of when repeating the STRmix™ analysis is appropriate include:

- An LR = 0 is obtained for a single locus, with other loci having an LR > 0, when the reference profile is consistent with the evidentiary profile. Review of the genotype probabilities may indicate that STRmix™ did not consider all potential genotype sets. Repeat analysis should be conducted with the same STRmix™ settings.
- An LR = 0 is obtained for a single locus, with other loci having an LR > 0, when the reference profile is consistent with the evidentiary profile. Review of the electropherogram indicates that an allele was not included in the STRmix™ analysis (e.g., a 9.3 allele that was not sufficiently resolved from a 10 allele). In this instance, the analysis should be repeated with the locus ignored in STRmix™.
- An observation that does not appear intuitively correct, such as (a) mixture proportions that do not reflect what is observed in the typing results, (b) degradation that does not reflect what is observed, or (c) the interpreted contributor genotypes do not appear intuitively correct.
- The number of contributors to a sample is ambiguous, or the STRmix™ results are not intuitively correct under the assumption of the tested number of contributors. Also, the number could be underestimated if the contributors are related and share alleles. The Examiner may repeat the STRmix™ analysis assuming a different number of contributors.

Should the diagnostics or results indicate that further scrutiny is required, a number of possible rework options are available. For example:

- Repeat STRmix™ analysis as appropriate. Should the original STRmix™ results be caused by a possible software limitation and subsequent results are acceptable, the initial STRmix™ results need not be included in the case file.
- Reassessment of the assumed number of contributors.
- Rerun or reamplification with the same or different amount of DNA template to strengthen the number of contributors assumption or assist with sub optimal PCR performance and/or allele designation.

5.4.8.3.1 If the STRmix™ analysis has been carried out more than once based on alternate propositions and/or assumptions, all analyses will be included in the case file, but the result of the most appropriate analysis in the context of the case[9] will be reported. The Examiner will determine which STRmix™ analysis to use for the reported conclusion.

5.4.8.3.1.1 A STRmix™ analysis may be rejected when a different STRmix™ analysis is preferred based upon the assessment of the diagnostics or based upon additional reviews of the electropherogram

[9] Case information available at the time of reporting is used to determine the most appropriate hypotheses and assumptions to include in the report.

data in the context of the case. A note will be added to the rejected STRmix™ analysis to describe the reason for the rejection.

5.4.8.3.1.2 When a STRmix™ analysis generates a result that does not appear intuitively correct and cannot be resolved as described above in 5.4.8.3, the examiner should consult with the TL for further guidance on reporting.

5.4.8.4 If a profile has been analyzed in STRmix™ with acceptable results and a subsequent analysis is performed, such as a comparison to a new reference profile, the subsequent analysis should be performed using the "LR from previous analysis" function in STRmix™.

5.4.9 Likelihood Ratio (LR)

A variety of LRs are provided in the STRmix™ results output, including those for relatives.

5.4.9.1 Generally, the unrelated highest posterior density (HPD) LR (with the factor of N! enabled) will be reported. However, if analysis in consideration of a relative of a POI is appropriate, the value for the relationship of interest may be reported with TL approval.

5.4.9.2 The settings for LR calculation are shown in Appendix B and Appendix F, to include the Factor of N! LR and the HPD with a one-sided quantile of 0.99.

5.4.9.3 LRs are calculated using four general United States population groups (African American, Caucasian, Southeastern Hispanic, and Southwestern Hispanic). Additional LRs will be calculated for specimens that potentially originate from Native American populations (i.e., Apache, Minnesota Native American, and Navajo), Caribbean populations (i.e., Trinidadian), or Chamorro/Filipino populations.[10]

5.4.9.3.1 The allele frequency distributions for the African American, Caucasian, Southeastern Hispanic, Southwestern Hispanic, Apache, Navajo, Trinidadian, Chamorro, and Filipino populations are published in *Forensic Science International: Genetics*.[11] The allele frequency distributions for the Minnesota Native American population are included in Appendix D. The African American population includes samples from the African American, Bahamian, and Jamaican populations.

5.4.9.3.2 At any locus, the published allele frequencies are incorporated into STRmix™ for each allele that contains a value. Refer to the STRmix™ User's Manual for information regarding alleles for which no frequency value is available.

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[10] The use of Native American, Caribbean, or Chamorro/Filipino population databases is generally based on the geographic location of the requesting agency. The listed Native American or Caribbean population databases are appropriate for use regardless of the specific Native American or Caribbean population group in the case scenario. Statistics for cases originating from Puerto Rico will be calculated using the four general United States population databases and do not require the use of the Caribbean population databases. The Chamorro/Filipino population databases would be used generally for cases originating from the U.S. territories of Guam and the Commonwealth of Northern Mariana Islands (e.g., Saipan).

[11] Moretti TR, Moreno LI, Smerick JB, Pignone ML, Hizon R, Buckleton JS, Bright J-A, Onorato AJ. Population data on the expanded CODIS core STR loci for eleven populations of significance for forensic DNA analyses in the United States. Forensic Science International: Genetics (2016) 25: 175-181.

5.4.9.4 The single lowest LR (HPD) obtained across all populations used in the calculation will be reported.

5.4.10 Determination of Typing Results Suitable for CODIS[12]

5.4.10.1 Forensic Unknown and Forensic Partial profiles may be entered into CODIS directly. Samples uploaded to the Forensic Mixture specimen category will be deconvoluted using STRmix™ to determine the CODIS profile. Generally, a particular STRmix™ contributor may be entered into CODIS if, at each given locus, the genotype(s) exhibit a (combined) weight of at least 99%. For example, if the STRmix™ results for Contributor 1 indicate the following genotypes and weights for a locus:

Contributor 1:

Genotype	Weighting
8, 8	41.31
8, 10	30.11
10, 10	24.51
7, 10	3.92
6, 8	0.15

The total weight of the first four genotypes is 99.85%. Therefore, "7, 8, 10" would be entered into CODIS for this locus. The 6 allele would not be entered. Alternatively, the genotypes for a particular STRmix™ contributor may be taken directly from the "SUMMARY>=99%" table that is a part of the STRmix™ output.[13]

5.4.10.2 In the event that the match rarity for a contributor does not meet the criteria for searching at a particular level for CODIS, the combined weight of the genotypes may be lowered to 95%. In the above example, at 95%, the "8, 10" would be entered into CODIS for this locus. The 6 and 7 alleles would not be entered.

5.5 Suggested Reporting Language

The results and/or conclusions for specimens subjected to DNA analysis will generally be reported in narrative form. The formatting and administrative information required in a report are described in the appropriate *FBI Laboratory Operations Manual* practices and *DNA Procedures Manual* procedures (i.e., DNA 610*)*. For additional guidance on reporting language for Y-STRs or familial comparisons refer to the appropriate interpretation protocols of the *DNA Procedures Manual*.

5.5.1 Introductory Statements

5.5.1.1 Items Analyzed and Amplification Kit(s) Used

The report must indicate (a) that the items were subjected to DNA typing, (b) that PCR methodology was used in DNA analysis, (c) specify which Amplification Kit was used, and (d) that the STRmix™ software was used, if applicable. The report should contain the item listing followed by the general

[12] DNA typing results may be entered into other appropriate databases.
[13] STRmix™ rounds weightings to determine whether a genotype is included in this table; it is possible that an included genotype will be just less than 99%. However, these genotypes are sufficient for CODIS purposes.

introductory statement:

> *"The items listed above were subjected to serological testing and/or nuclear deoxyribonucleic acid (DNA) analysis.* Probabilistic genotyping was performed using the STRmix™ software."*

With the appropriate associated endnote:

> *"* DNA typing using the polymerase chain reaction (PCR) of short tandem repeats (STRs) was performed with the GlobalFiler™ PCR Amplification Kit."*
>
> *"* DNA analysis using the polymerase chain reaction (PCR) was performed with the Quantifiler™ Trio DNA Quantification Kit."*

If the DNA typing results will be included in the report, they should generally be included as follows:

> *"The DNA typing results are detailed below:"*

Locus	**JONES**
D3S1358	15, 16
vWA	14, 16
D16S539	9, 10
CSF1PO	11, 12
TPOX	8
Y indel	2
Amelogenin	X, Y
D8S1179	12, 13
D21S11	28, 31
D18S51	12, 15
DYS391	11
D2S441	14, 15
D19S433	14, 15
TH01	7, 9.3
FGA	24, 26
D22S1045	11, 16
D5S818	11
D13S317	11
D7S820	7, 12
SE33	17, 25.2
D10S1248	12, 15
D1S1656	13, 16
D12S391	18, 19
D2S1338	20, 23

5.5.1.2 Comparisons to Previously Reported Results

Generally, one of the following statements should be included under the results section of the report to identify item(s) previously subjected to DNA typing but for which the results of additional comparisons are being reported:

> *"The DNA typing results from items 1 and 2 were compared to the DNA typing results from SMITH [previously reported under FBI Laboratory Number 2020-01234-2 in the report dated July 26, 2020]. The results for JONES were also included in the report dated July 26, 2020."*
>
> *"The DNA typing results from SMITH were compared to the DNA typing results from item 1 [previously reported under FBI Laboratory Number 2020-01234-1, FBI Case Identification number 95A-HQ-0123456, in the report dated July 26, 2020]."*
>
> *"Items 1, 2, and 7 (JONES) were previously reported under FBI Laboratory Report 2020-01234-2 on July 26, 2020. The DNA typing results from SMITH were compared to the DNA typing results from items 1 and 2. The conclusions reported for items 1 and 2, in the report dated July 26, 2020, are being amended as a result of implementing new interpretation guidelines that utilize probabilistic genotyping software."*
>
> *"The DNA typing results from the items listed above were compared to the DNA typing results from SMITH [previously reported under FBI Laboratory Number 2020-01234-2 in the report dated July 26, 2020]."*
>
> *"The DNA typing results from items 1 and 2 were compared to the DNA typing results from SMITH [provided by the New Jersey State Police Office of Forensic Sciences on December 16, 2019]."*

5.5.1.3 Alternate Reference Samples

The use of a questioned item of established origin as an alternate reference sample should generally be stipulated in the report as follows:

> *"It is noted that for comparison purposes, item 1 is being used as an alternate reference sample for JOHN SMITH."*
>
> *"Per incoming communication dated November 20, 2020 from Special Agent Jane Doe, item 7 (shirt) is being used as an alternate reference sample for JOHN SMITH."*
>
> *"Per communication with Special Agent Jane Doe on November 20, 2020, item 7 (shirt) is being used as an alternate reference sample for JOHN SMITH."*
>
> *"Assuming the major contributor to item 1 is JOHN SMITH, the DNA profile of the major contributor to item 1 is being used as an alternate reference sample for JOHN SMITH."*

5.5.1.4 Elimination Samples

The use of a reference item identified by the contributor as an elimination sample, such as a consensual partner or an evidence technician, should generally be captured in the report as follows:

> *"Per incoming communication dated May 15, 2020 from SA Michael Jones, BROWN was provided as an elimination sample for comparison to items 3 through 10."*

The elimination sample should be used for comparison and reporting if:

- There is no DNA unlike the elimination sample.
- The elimination sample is included and is conditioned upon for STRmix™ purposes.
- The elimination sample is visually excluded.

If based on the results, the elimination sample is not used for conditioning and no visual exclusion is possible, additional language should be added:

> *However, based on the nature of the results, BROWN was not required for the interpretation of items 4 and 8.*

This additional language is not needed if no results are obtained for an item.

5.5.1.5 Relationships

For missing person reports, a statement should generally be included to define the relationships of the submitted reference samples to the missing person as follows:

> *"It is noted that SARAH SMITH is identified by the incoming communication from the contributor as the biological mother of the missing person JANE SMITH."*

5.5.1.6 Differentially Extracted Samples

5.5.1.6.1 The designations 1F and 1M may be truncated to the original item identifier (e.g., item 1) for reporting purposes. Differentially extracted samples that result in distinct fractions may be reported together where the fractions are treated as individual contributors to a mixture. This approach may also be used if the DNA typing results from both fractions are the same. DNA types consistent with carry-over from either fraction may be subtracted from the opposite fraction by using the profile as a conditional reference sample in STRmix™.

5.5.1.6.2 The designations 1F and 1M may be maintained if it is necessary to report the DNA typing results from each fraction separately. An explanatory statement should be included in the report as follows:

> *"Item 1 was extracted in two fractions, which will be designated items 1M and 1F."*

5.5.2 Reporting STRmix™ Results and Conclusions

5.5.2.1 Each DNA association must be clearly and properly qualified with either a statistic or a qualitative statement. STRmix™ analysis is performed to provide a statistic. A qualitative statement

not based on a statistical calculation should be limited to situations in which the presence of an individual's DNA on an item is reasonably expected. The provenance of the sample must be established in the case record when statistics are not calculated.

5.5.2.2 The sex typing results for a sample should be reported based on the three sex determining loci: amelogenin, Y indel and DYS391 (see 5.4.3).

5.5.2.3 For a given STRmix™ analysis, the assumption as to the number of contributors (N) and any individuals assumed to be present in the sample (conditioned contributors) should be reported. The assumed number of contributors in the report should match the STRmix™ analysis.

5.5.2.4 The single lowest HPD LR value across all appropriate populations is used for drawing conclusions, and it is generally truncated to two significant digits for reporting. However, if this value is between 1/10 and 10, it is truncated to one significant digit for reporting. For $1/100 < LR < 1$ (i.e., $0.01 < LR < 1$), the reciprocal should be calculated prior to truncating for reporting.

5.5.2.5 The magnitude of the LR relates to the degree of support provided by the evidence under the tested hypotheses and assumptions. A qualitative statement should be reported based on the following table:

LR	Qualitative Equivalent
0 to 1/100	Exclusion
>1/100 to 1/2	Limited support for Exclusion
1	Uninformative
2 to <100	Limited support for Inclusion
100 to <10,000	Moderate support for Inclusion
10,000 to <1,000,000	Strong support for Inclusion
≥ 1,000,000	Very strong support for Inclusion

5.5.2.6 LRs >1/100 (i.e., 0.01) will have a statement included in the report and should also be reported in tabular form, except when LR=1. The table should include the name of the POI(s), any assumed contributors, the LR, and the qualitative level of support for the conclusion.

5.5.2.7 All exclusions, visual exclusions and LR ≤ 1/100 (i.e., 0.01), may be reported using a bulleted list of the excluded POI(s). The LR value need not be reported for exclusions.

5.5.2.8 Report Wording Examples

Results and conclusions are generally preceded by a description of the item tested and/or, where appropriate, the sampling area tested from an item.

See section 5.5.2.9 for endnote language, denoted A-E in these examples.

Item 1 (Pants from Jones)

Item 1(1) (Bloodstain from pants)

Male DNA is present in item 1(1). Item 1(1) was interpreted as originating from one individual.

The DNA results from item 1(1) are 74 sextillion times more likely if JAMES is a contributor than if an unknown, unrelated person is a contributor.

Person of Interest (POI)	Likelihood Ratio (LR)[A]	Level of Support[B]
JAMES	7.4×10^{22} (74 sextillion)	Very strong support for **Inclusion**

The following individuals are excluded as potential contributors to item 1(1):[C]

- GARCIA
- JONES
- WHITE

Item 1(3) (Stain from pants) **[support for H_2 changes the sentence before the table]**

No conclusion regarding sex typing results can be provided for item 1(3). Item 1(3) was interpreted as originating from two individuals.

The DNA results from item 1(3) are 40 times more likely if two unknown, unrelated people are contributors than if JAMES and an unknown, unrelated person are contributors.

Person of Interest (POI)	1/Likelihood Ratio (1/LR)[A]	Level of Support[B]
JAMES	40	Limited support for **Exclusion**

The following individuals are excluded as potential contributors to item 1(3):[C]

- GARCIA
- JONES
- WHITE

Item 1(5) (Stain from pants) **[no table for LR=1]**

Male DNA[D] is present in item 1(5). Item 1(5) was interpreted as originating from two individuals.

The DNA results from item 1(5) are equally likely if JONES and an unknown, unrelated person are contributors than if two unknown, unrelated people are contributors.[E]
The following individuals are excluded as potential contributors to item 1(5):[C]

- GARCIA
- JAMES
- WHITE

Item 3 (Swab of wall) [if all have same (H_1 or H_2) support, enter both in the table together and use placeholders; LR=1 for >1 POI]

Male and female DNA was obtained from item 3. Item 3 was interpreted as originating from three individuals.

The DNA results from item 3 are **[LR]** times more likely if **[POI]** and two unknown, unrelated people are contributors than if three unknown, unrelated people are contributors.

Person of Interest (POI)	Likelihood Ratio (LR)[A]	Level of Support[B]
JAMES	2.6×10^4 (26,000)	Strong support for **Inclusion**
GARCIA	3.4×10^7 (34 million)	Very strong support for **Inclusion**

The DNA results from item 3 are equally likely if any of the following individuals and two unknown, unrelated people are contributors than if three unknown, unrelated people are contributors.[E]

- JONES
- WHITE

Item 3-1 (Swab of wall) [>1 POI in table, all H_2 support; the sentence changes]

Male and female DNA was obtained from item 3-1. Item 3-1 was interpreted as originating from three individuals.

The DNA results from item 3-1 are **[1/LR]** times more likely if three unknown, unrelated people are contributors than if **[POI]** and two unknown, unrelated people are contributors.

Person of Interest (POI)	1/Likelihood Ratio (1/LR)[A]	Level of Support[B]
JAMES	83	Limited support for **Exclusion**
GARCIA	22	Limited support for **Exclusion**

The following individuals are excluded as potential contributors to item 3-1:[C]

- JONES
- WHITE

Item 3-2 (Swab of wall) [different support gets separate table; if >1 POI in a table, then use placeholders in the sentence before]

Male and female DNA was obtained from item 3-2. Item 3-2 was interpreted as originating from three individuals.

The DNA results from item 3-2 are 26,000 times more likely if JAMES and two unknown, unrelated people are contributors than if three unknown, unrelated people are contributors.

Person of Interest (POI)	Likelihood Ratio (LR)[A]	Level of Support[B]
JAMES	2.6×10^4 (26,000)	Strong support for **Inclusion**

The DNA results from item 3-2 are 75 times more likely if three unknown, unrelated people are contributors than if GARCIA and two unknown, unrelated people are contributors.

Person of Interest (POI)	1/Likelihood Ratio (1/LR)[A]	Level of Support[B]
GARCIA	75	Limited support for **Exclusion**

The DNA results from item 3-2 are equally likely if any of the following individuals and two unknown, unrelated people are contributors than if three unknown, unrelated people are contributors.[E]

- JONES
- WHITE

<u>Item 4 (neck swab)</u> [e.g., 2 person mix assuming White; same if differential extraction and assuming in Male (M) fraction; H_2 support]

Male and female DNA was obtained from item 4. Item 4 was interpreted as originating from two individuals, one of whom is WHITE.

The DNA results from item 4 are 45 times more likely if WHITE and an unknown, unrelated person are contributors than if WHITE and GARCIA are contributors.

Person of Interest (POI)	Assumed Contributor	1/Likelihood Ratio (1/LR)[A]	Level of Support[B]
GARCIA	WHITE	45	Limited support for **Exclusion**

The DNA results from item 4 are equally likely if WHITE and JONES are contributors than if WHITE and an unknown, unrelated person are contributors.[E]

JAMES is excluded as a potential contributor to item 4.[C]

<u>Item 5 (vaginal swab)</u> [e.g., clean male in M fraction; clean or mixed female in Female (F) fraction; STRmix™ on the single source clean male]

Male and female DNA was obtained from item 5. Item 5 was interpreted as originating from two individuals, one of whom is WHITE.

The DNA results from item 5 are 8.6 billion times more likely if GARCIA is a contributor than if an unknown, unrelated person is a contributor.

Person of Interest (POI)	Likelihood Ratio (LR)[A]	Level of Support[B]
GARCIA	8.6×10^9 (8.6 billion)	Very strong support for **Inclusion**

The following individuals are excluded as potential contributors to item 5:[C]

- JAMES
- JONES

<u>Item 6 (rectal swab)</u> [e.g., clean F fraction, 2 person mix in M, no indication of a 3rd person, but NOT assuming White in STRmix™ – limited to intimate samples (e.g., fingernail samples, neck swabs)]

Male and female DNA was obtained from item 6. Item 6 was interpreted as originating from two individuals, one of whom is WHITE.

The DNA results from item 6 are 8.6 billion times more likely if GARCIA and an unknown, unrelated individual are contributors than if two unknown, unrelated people are contributors.

Person of Interest (POI)	Likelihood Ratio (LR)[A]	Level of Support[B]
GARCIA	8.6×10^9 (8.6 billion)	Very strong support for **Inclusion**

The following individuals are excluded as potential contributors to item 6:[C]

- JONES
- JAMES

<u>Item 7 (cervical swab)</u> [e.g., clean F fraction matches White; 2 person mix in M fraction, but 2nd person is not White; 3 people overall (only difference between 6 and 7 is second sentence)]

Male and female DNA was obtained from item 7. Item 7 was interpreted as originating from three individuals, one of whom is WHITE.

The DNA results from item 7 are 37 billion times more likely if GARCIA and an unknown, unrelated individual are contributors than if two unknown, unrelated people are contributors.

Person of Interest (POI)	Likelihood Ratio (LR)[A]	Level of Support[B]
GARCIA	3.7×10^{10} (37 billion)	Very strong support for **Inclusion**

The following individuals are excluded as potential contributors to item 7:[C]

- JONES
- JAMES

<u>Item 10 (Blanket)</u> [e.g., clean M fraction matches Garcia; F fraction is a 2 person mix of M fraction profile and White; reporting separate fractions]

<u>Item 10(1) (Semen stain from blanket)</u>

Item 10(1) was extracted in two fractions, which will be designated items 10(1)M and 10(1)F.

Male DNA was obtained from item 10(1)M. Item 10(1)M was interpreted as originating from one individual.

The DNA results from item 10(1)M are 21 quintillion times more likely if GARCIA is a contributor than if an unknown, unrelated person is a contributor.

Person of Interest (POI)	Likelihood Ratio (LR)[A]	Level of Support[B]
GARCIA	2.1×10^{18} (2.1 quintillion)	Very strong support for **Inclusion**

Male DNA[D] was obtained from item 10(1)F. Item 10(1)F was interpreted as originating from two individuals, one of whom is the male obtained from item 10(1)M.

The DNA results from item 10(1)F are 450 quintillion times more likely if the male from 10(1)M and WHITE are contributors than if the male from 10(1)M and an unknown, unrelated person are contributors.

Person of Interest (POI)	Assumed contributor	Likelihood Ratio (LR)[A]	Level of Support[B]
WHITE	Male from 10(1)M	4.5×10^{20} (450 quintillion)	Very strong support for **Inclusion**

The following individuals are excluded as potential contributors to item 10(1):[C]

- JONES
- JAMES

5.5.2.9 Associated Endnotes for Reporting Language

The following endnotes should generally be used for reporting.

[A] The likelihood ratio is a statistical approach that compares the probabilities of observing the DNA results under two alternative propositions. Calculations were performed using the African American, Caucasian, Southeastern Hispanic, and Southwestern Hispanic populations. The lowest calculated likelihood ratio is reported.

[B] These likelihood ratio ranges provide the following support for the conclusion:

Likelihood Ratios:	Qualitative Equivalent:
≤ 1/100	**Exclusion**
>1/100 to 1/2	Limited support for **Exclusion**
1	Uninformative
2 to <100	Limited support for **Inclusion**
100 to <10,000	Moderate support for **Inclusion**
10,000 to <1,000,000	Strong support for **Inclusion**
≥1,000,000	Very strong support for **Inclusion**

[C] A person of interest is excluded either visually or when the likelihood ratio is less than or equal to 1/100. An exclusion means that the person of interest was not detected in the DNA results.

[D] The presence of male DNA in a mixture may limit the ability to determine if female DNA is also present in that mixture.

[E] This conclusion is drawn when the likelihood ratio is equal to 1; this comparison is uninformative.

5.5.2.10 Terrorist Explosive Device Analytical Center (TEDAC) Report Wording Example

The following example is the formatting and report wording used when reporting results obtained from TEDAC specimens subjected to DNA analysis. These results are generally reported in tabular form, with the associated endnotes F and G, as follows.

Item(s)	Profile suitable for comparison purposes [F]	Database Entry [G]
Item 1	YES	YES
Item 2	NO	
Item 3	NR	

[F] The results listed in the table indicate if a DNA profile was generated that is suitable for comparison purposes. NR means that no STR results were obtained from that sample. NO means that a mixture of five or more individuals was obtained and the results are not suitable for comparisons. YES means that the results obtained from that sample are suitable for comparison purposes.

[G] The eligible DNA results for the samples marked YES will be entered into the Combined DNA Index System (CODIS) and/or any other appropriate database. When the same or similar DNA typing results are obtained from multiple items, only the item that yields the most complete profile is entered. These results will be maintained by the FBI Laboratory for possible future comparisons.

5.5.3 Unsuitable Results

Mixtures of DNA from 5 or more contributors, will be reported as unsuitable for analysis. Any comparison declared unsuitable for analysis should generally be reported as follows:

"A mixture of five or more individuals was obtained from item 1; therefore, the results are not suitable for comparisons."

5.5.4 No DNA Results

For samples for which no DNA typing results were obtained, this information should be reported as follows:

"No DNA typing results were obtained from item 1; therefore, no comparisons can be made."*

"Male (Female) DNA was obtained from item 1. No additional DNA typing results were obtained from item 1; therefore, no comparisons can be made."*

"No DNA was detected from item 1; therefore, no DNA typing was conducted."*

with the following endnote:

*"*Insufficient DNA quality and/or quantity can affect the ability to generate a result and is not an absolute determination that an individual did not come into contact with an item of evidence."*

5.5.5 No DNA Results other than an expected contributor

For samples for which no DNA typing results other than an expected contributor were obtained, this information should be reported as follows:

Female DNA is present in item 1. No DNA typing results unlike SMITH were obtained from item 1.

Male DNA is present in item 5. No DNA typing results unlike JONES were obtained from item 5. Therefore, no comparisons were made to GREEN.

5.5.6 Unknown Subject (UNSUB) Results

If reference samples are not submitted, or if the evidentiary profile does not match any reference samples, the results may be reported as follows along with the appropriate exclusionary statement:

"Item 1 is consistent with originating from a single male (female) individual and is suitable for comparison purposes."

"The mixture of DNA obtained from item 3 contains female DNA and is suitable for comparison purposes."

"The mixture of DNA obtained from item 4 contains male DNA and is suitable for comparison purposes.*

** The presence of male DNA in a mixture may limit the ability to determine if female DNA is also present in that mixture."*

"The mixture of DNA obtained from item 5 contains male and female DNA and is suitable for comparison purposes."

"Male and female (Male; Female) DNA was obtained from item 6. The DNA unlike JONES obtained from item 6 is consistent with originating from a single male (female) individual and is suitable for comparison purposes."

"No conclusion regarding sex typing results can be provided for item 7. Item 7 is consistent with originating from a single individual (mixture of individuals) and is suitable for comparison purposes."

5.5.7 Contamination

The following wording can be used as a guide to report contamination in the event the samples are not or cannot be reprocessed.

Random contamination:

"The negative control that was processed together with item 1 displayed possible contamination. No sample remains from item 1 for retesting; however, because the DNA results from the possible contaminant are not present in item 1, and therefore not systemic in nature, the DNA results for item 1 were used for comparison purposes."

"DNA consistent with laboratory staff was present in the DNA obtained from item 1. This individual was involved in the processing or handling of item 1, and retesting was not possible. Therefore, this individual was treated as an assumed contributor to the DNA obtained from item 1 and will further be referred to as STAFF."

Systemic contamination:

"The negative control that was processed together with item 1 displayed possible contamination. The DNA typing results obtained from the negative control were observed in one or more samples or controls and therefore, may be systemic."

The systemic contamination statement may be supplemented with the wording below depending upon the specific situation:

"No sample remains from item 1 for retesting; therefore, no conclusion can be offered in regard to item 1."

"Item 1 was not reprocessed per communication with SA James Madison on DATE; therefore, no conclusion can be offered in regard to item 1."

"No sample remains from item 1 for retesting; however, no DNA foreign to SMITH was obtained from item 1."

"No sample remains from item 1 for retesting; however, no DNA was obtained from item 1."

5.5.8 No other examinations

A statement that no additional serology or nuclear DNA examinations were conducted should be made.

5.5.9 CODIS Statements

A statement must be included in the report of DNA examinations that indicates when an item's typing results will be initially entered into the CODIS or other appropriate database. Alternatively, a statement is typically added when no results are entered into CODIS. This information should generally be reported as follows; however, TEDAC reports may address database entry in the tabular report format (see 5.5.2.10).

CODIS and/or other database entry:

"The eligible DNA typing results for item 1 will be entered into the Combined DNA Index system (CODIS)."

"The DNA typing results for item 1 will be entered into the (Unidentified Human Remains/Missing Persons/Relatives of Missing Persons) Index of the Combined DNA Index system (CODIS)."

"The DNA typing results obtained from the tested items are not eligible for entry into the Combined DNA Index System (CODIS); however, the results from SMITH will be maintained and searched in the appropriate databases."

No CODIS entry:

"The DNA results obtained from the tested items are not eligible for entry into the Combined DNA Index System (CODIS).

"Because no DNA typing results were obtained, the tested items are not eligible for entry into the Combined DNA Index System (CODIS)."

5.5.10 Remarks Statements

5.5.10.1 A statement regarding the maintenance of results should be included. For example:

"These results will be maintained by the FBI Laboratory for possible future comparisons."

5.5.10.2 Request for Reference Samples:

If reference samples are requested, a note may be added:

"If future comparisons are requested, a known blood or buccal (saliva) sample from the victim/subject should be submitted."

5.5.10.3 Discontinued

At the request of a contributor, examinations may be discontinued after they have been initiated, up to the point that they are loaded onto the 3500xL. If examinations are discontinued, items should be reported, with the reason for the discontinuation included (in this example, a plea agreement), as follows:

"*Examinations were initiated on items 1 and 2 and were discontinued on the submitted item(s) per communication with Special Agent Smith on January 20, 2020, due to a plea agreement."*

5.5.10.4 Items Not Examined

If items were included in the evidence listing but not examined, the following note should be added:

"Items 3, 5, and 8 were not examined."

5.5.10.5 Consumption of Evidence

If items were consumed in the course of examinations, the following note should be added:

"Items 1, 2, and 4 were consumed during the DNA Casework Unit examinations."

5.5.10.6 Evidence Pending Examination

If additional items are pending examination and will be the subject of another report, the following note should be added:

"*Additional evidence is pending serological and/or DNA examinations and will be the subject of a separate report."*

6 Limitations

6.1 It is not possible to anticipate the nature of all potential DNA typing results or the nature of the evidentiary specimens from which they may be obtained. These procedures do not exhaust the possible list of the results that may be encountered by the Examiner, nor the conclusions that an Examiner may render based on his/her interpretation of those results. For those results not specifically described, conclusions should be drawn using the procedures given for the results above that are similar in concept and/or origin.

6.2 All reasonable attempts are made by DNA personnel to preserve material (i.e., evidence material and/or isolated DNA) for potential future DNA testing. However, it is sometimes necessary to consume a sample (e.g., a small bloodstain) in its entirety (i.e., no original material or isolated DNA

would remain) to ensure that the best attempt possible is made to obtain DNA typing results for comparison purposes. Should the total consumption of a sample be anticipated by an Examiner, or should the performance of any additional examination(s) result in the total consumption of a sample, an Examiner should ensure that the contributing agency or other responsible office (e.g., Office of the United States Attorney) is contacted concerning this necessity. Based on this discussion, a mutually acceptable strategy should be developed concerning the future testing of such a sample(s).

6.3 In DNA mixtures of closely related individuals (e.g., parents, offspring, and siblings), false inclusions of other closely related family members may occur due to the elevated sharing of genetic information between relatives.

6.4 Mixtures of DNA from 5 or more contributors will not be interpreted using STRmix™, and will be reported as unsuitable for comparisons.

6.5 Loci exhibiting a tri-allelic pattern cannot be assessed using the STRmix™ software. These loci will be ignored during STRmix™ analysis using the "ignore locus" function.

6.6 The presence of male DNA in a mixture may limit the ability to determine if female DNA is also present in that mixture.

6.7 Insufficient DNA quality and/or quantity can affect the ability to generate a DNA typing result and is not an absolute determination that an individual did not come into contact with an item of evidence.

6.8 LR calculations are based on allele frequency estimates from a sampling of each reported population. Uncertainty in these estimates, as well as uncertainties in population composition, F_{ST}, STRmix™ modeling, and MCMC are all accounted for by reporting the HPD with a one-sided quantile of 0.99 and incorporating the factor of N!. All reasonable uncertainty is conceded.

7 Calculations

Not applicable.

8 Measurement Uncertainty

Not applicable.

9 Sampling

The examiner will ensure the results in the *Laboratory Report* relate only to the items tested or sampled.

9.1 A reasonable assumption of homogeneity can be made for reference samples and various types of evidence examined by the DNA Units. This type of evidence includes known samples (e.g., blood tubes, buccal samples), bones, teeth, hair and swabs.

9.2 When a reasonable assumption of homogeneity cannot be assumed, the *Laboratory Report* will reflect the tested portion of the item of evidence, making no inference about the whole.

10 Safety

Not applicable.

11 References

FBI Laboratory Quality Assurance Manual

FBI Laboratory Operations Manual

DNA Procedures Manual

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Rev. #	Issue Date	History
4	03/16/20	1,4.4.b, 5.2.1.2, 5.4, 5.4.5.1, 5.5.10.3: revised for clarity 3: updated GMIDX version 5.1.1: Added reference to SOPs for ID+ GMIDX settings 5.1.1.1.1: updated stutter table based on additional validation studies 5.1.1.1.4: consolidated and updated spike guidance 5.1.1.1.6: added additional information re: artifacts 5.4.3: new criteria for sex determination implemented 5.4.6.1, 5.4.9.2: added reference to Appendix F. 5.4.7.3.4: removed "in the mixture" to make applicable to single source profiles 5.4.8.2: added separate tri-allele guidance for evidentiary and reference samples 5.5: added reference for DNA procedure 5.5.1.1, 5.5.4: language updated for Trio implementation 5.5.1.2: added example when a previous reference sample was reported, added additional examples 5.5.1.4: removed "at this time" from the example language 5.5.2.4: corrected and clarified based on our practice 5.5.2.8: added sampling description statement 5.5.9: removed requirement to add a statement when no results are entered into CODIS 9: added reporting guidance for sampling 11: added a reference Appendix A, Figures 2 and 3: added statement about adjusting Pt Appendix A, 7: new criteria for sex determination to mirror 5.4.3 Appendix E, changed title to Interpretation, added reference to 229, added requirement for authorization to reinterpret, moved ID+ STRmix settings to Appendix F
5	04/15/21	5.4.7.3.4: Added together to report language example 5.4.8.3.1: Added additional guidance for when multiple STRmix™ analyses are performed. 5.5.10.4 Deleted additional analysis statement.

Redacted - Signatures on File

Approval

DNA Technical Leader Date: 04/14/2021

DCU Chief Date: 04/14/2021

SBAU Chief Date: 04/14/2021

Appendix A: *GlobalFiler™ GMIDX Analysis Settings*

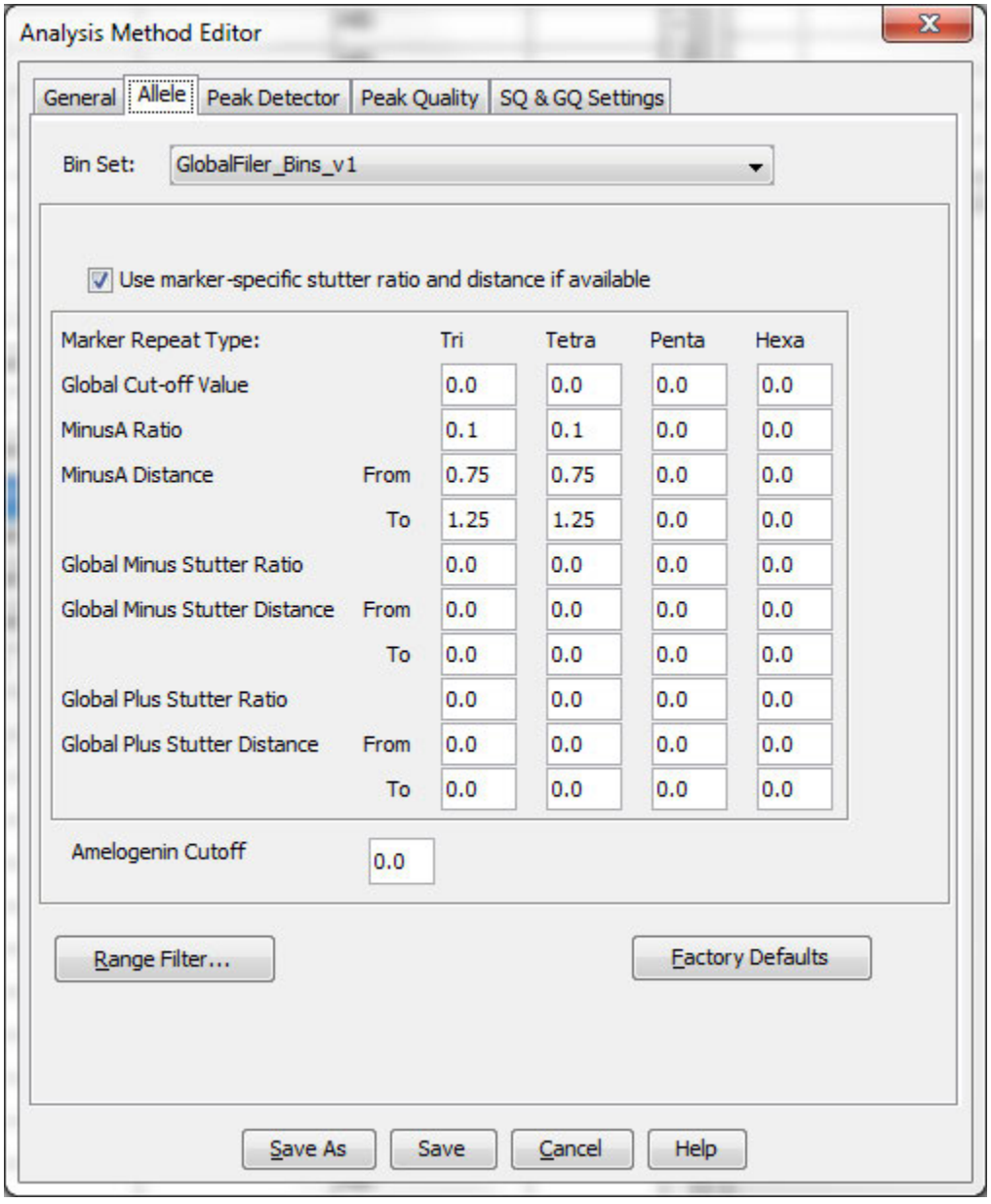


Figure 1 - Allele Tab for GlobalFiler™ 150AT and 50AT

Appendix A: *GlobalFiler™ GMIDX Analysis Settings (cont.)*

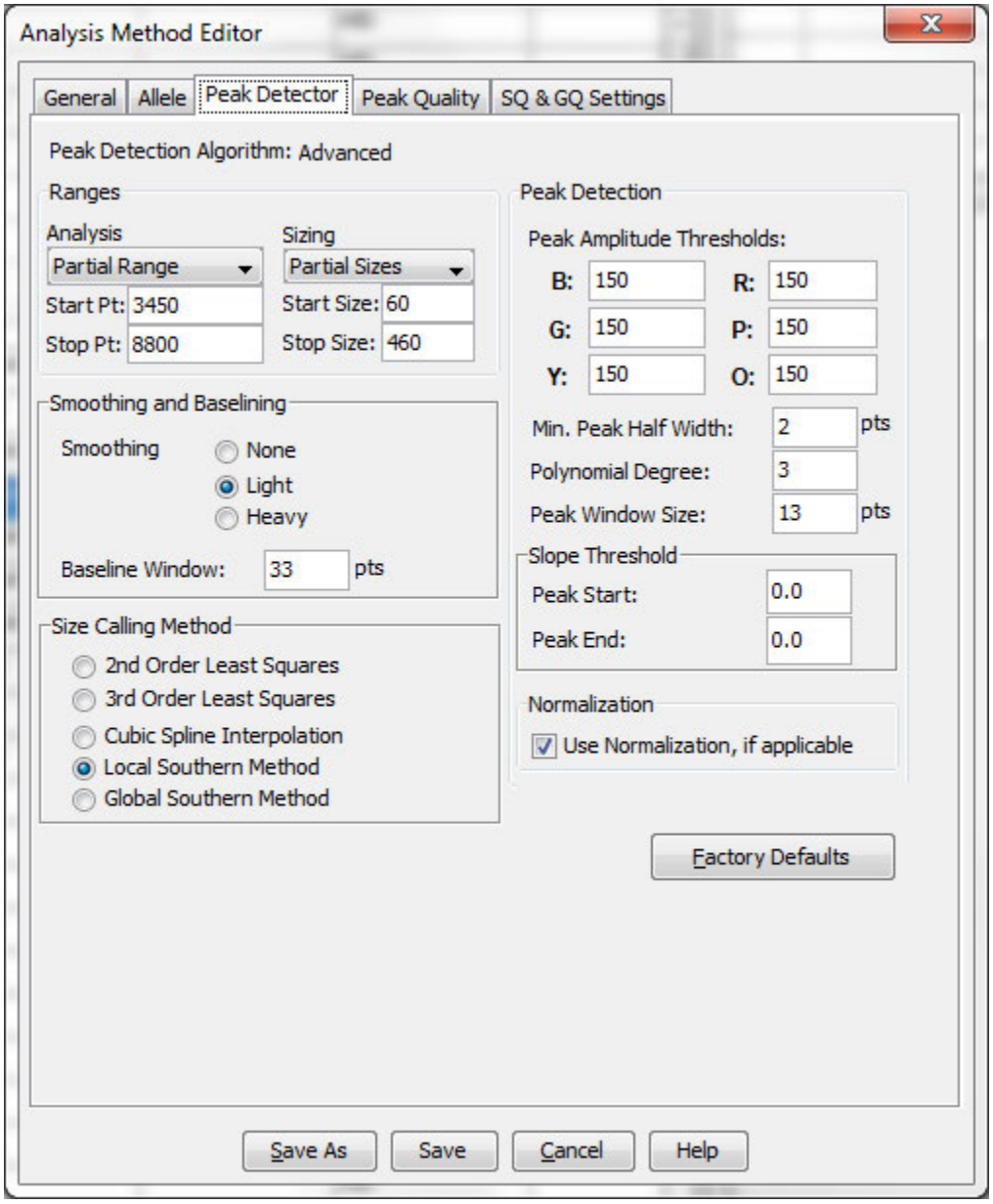


Figure 2 - Peak Detector Tab for GlobalFiler™ 150AT
Note: Analysis "Start Pt" and "Stop Pt" may be adjusted as needed

Appendix A: *GlobalFiler™ GMIDX Analysis Settings (cont.)*

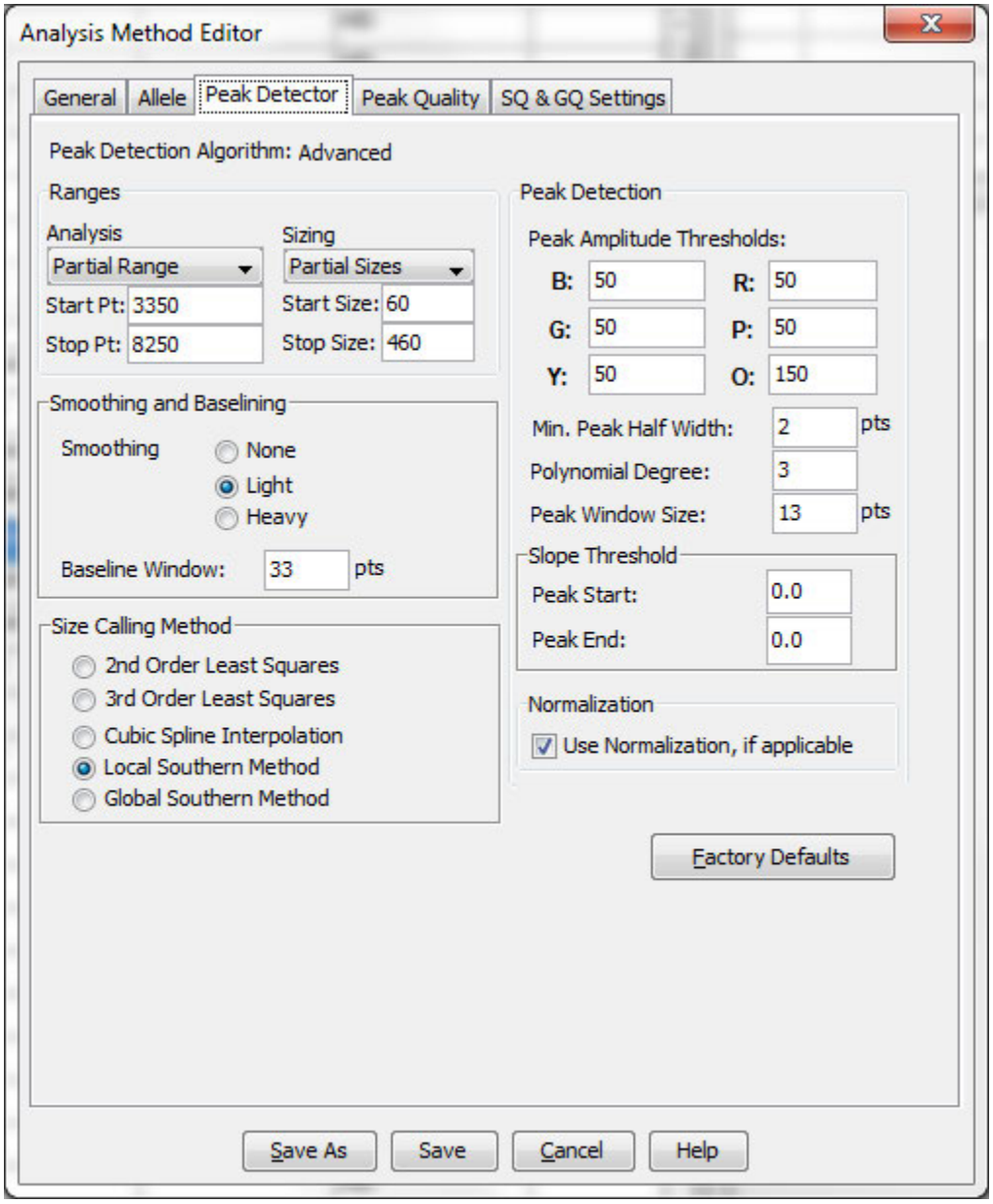


Figure 3 - Peak Detector Tab for GlobalFiler™ 50AT
Note: Analysis "Start Pt" and "Stop Pt" may be adjusted as needed

Appendix B: *GlobalFiler™ STRmix™ Analysis Settings*

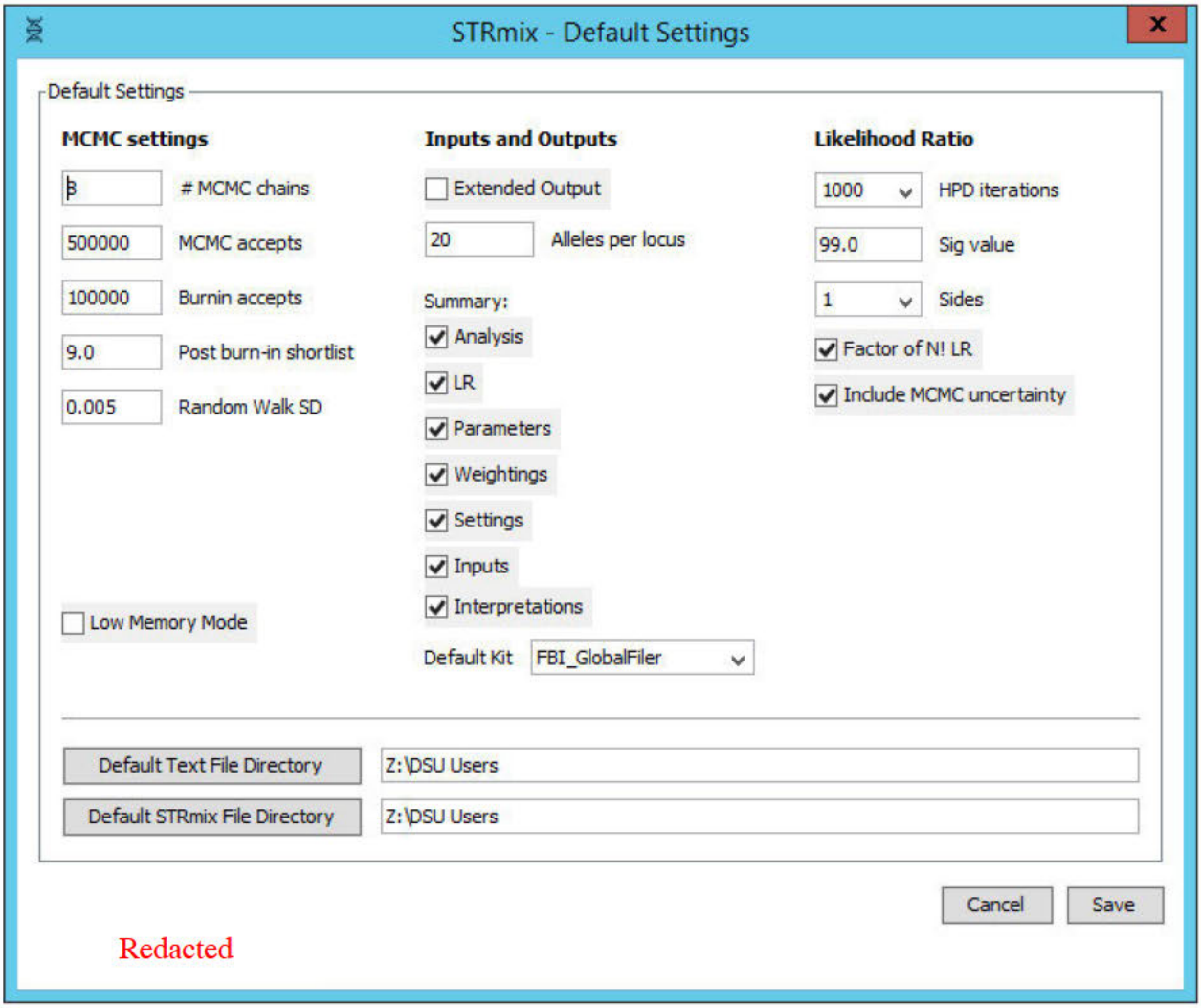


Figure 1 – STRmix™ V2.4.05 default settings

Appendix B: *GlobalFiler™ STRmix™ Analysis Settings (cont.)*

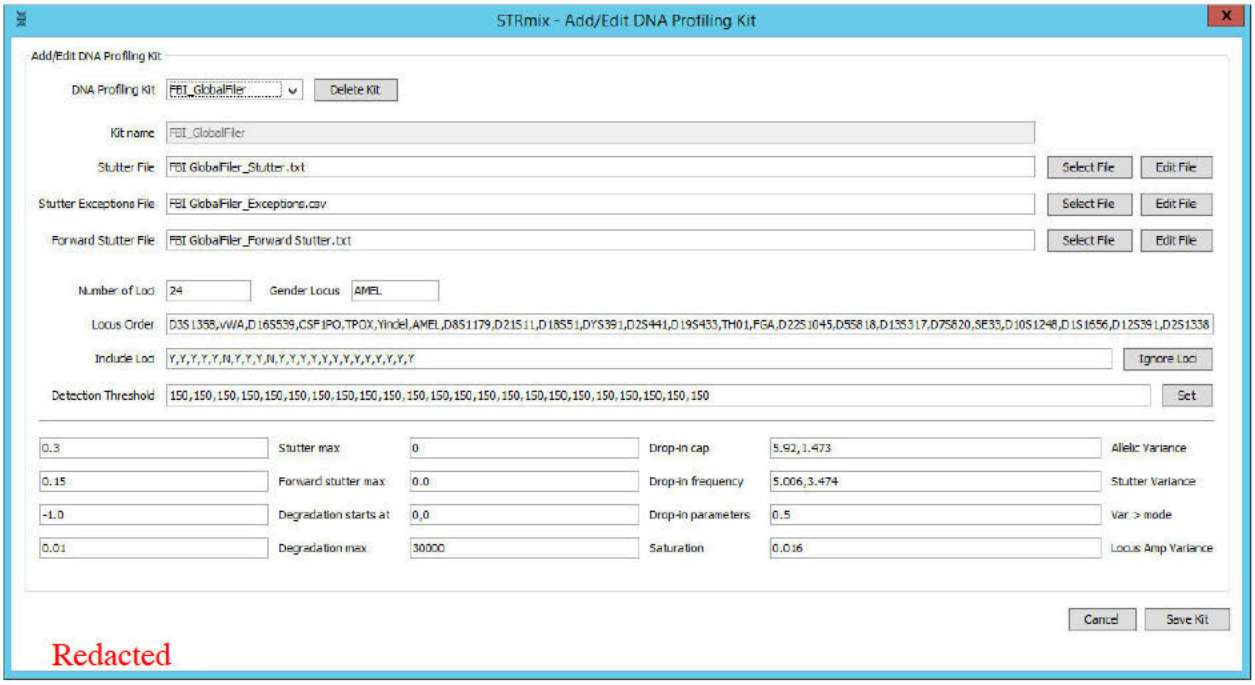


Figure 2 – GlobalFiler™ 150AT kit settings

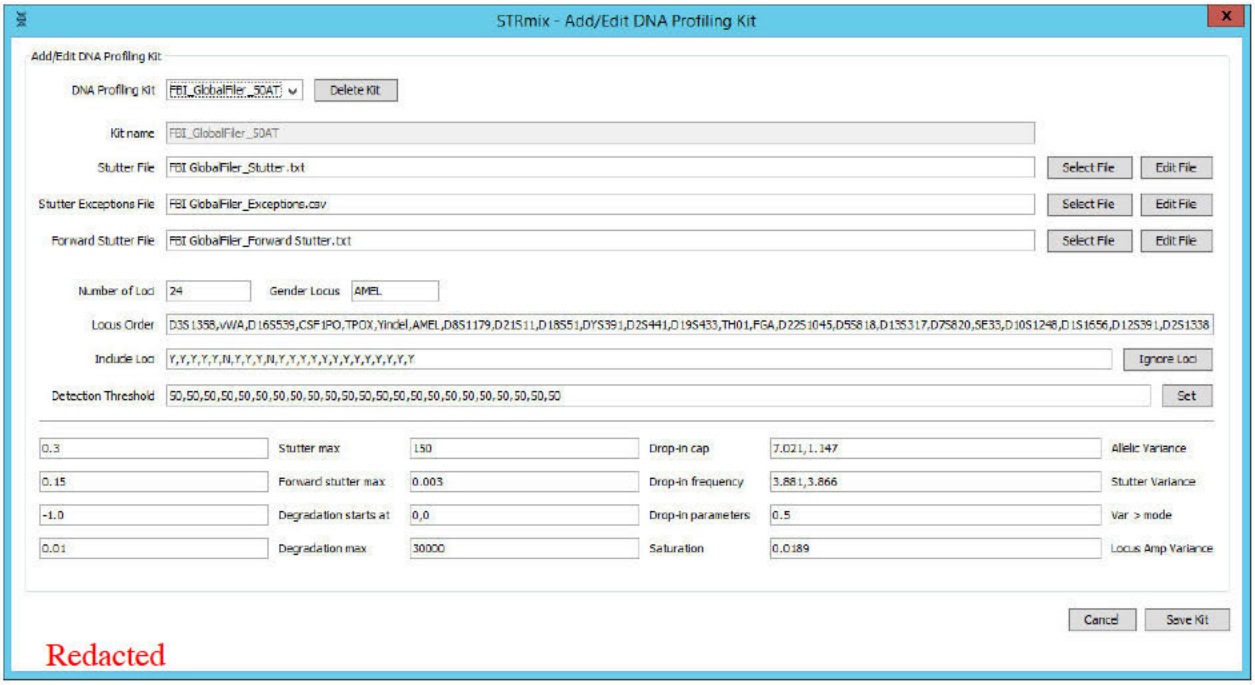


Figure 3 – GlobalFiler™ 50AT kit settings

Appendix B: *GlobalFiler™ STRmix™ Analysis Settings (cont.)*

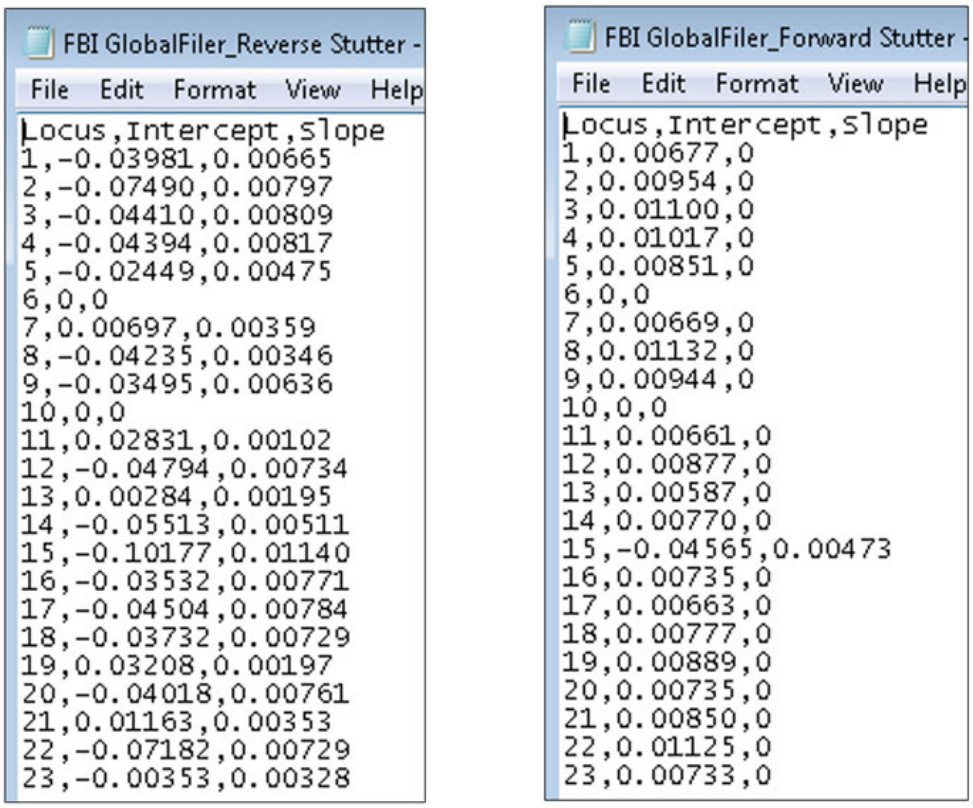
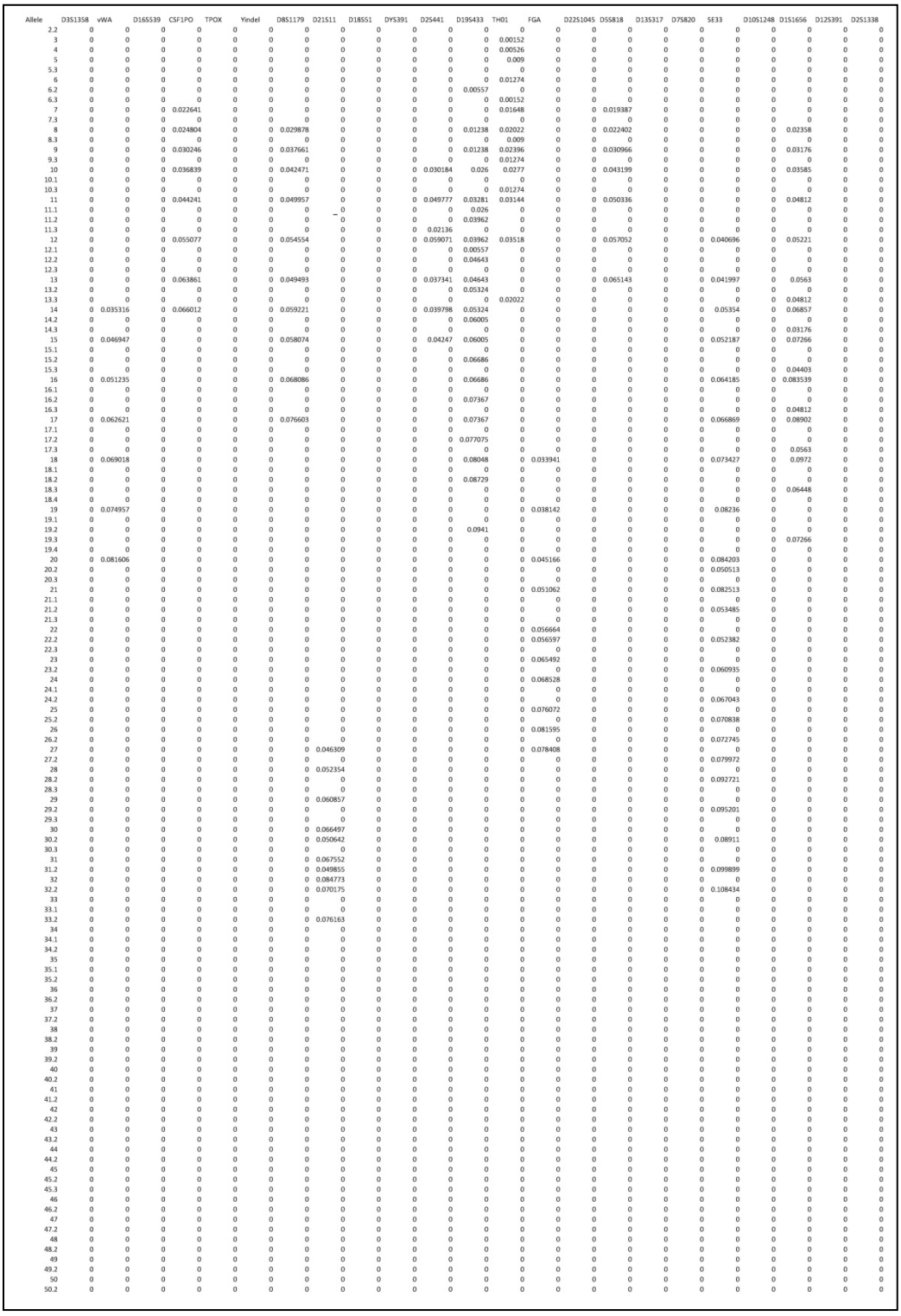


Figure 4 – Parameters used in determination of allele-specific stutter ratios at GlobalFiler™ loci (150AT and 50AT)

Appendix B: *GlobalFiler™ STRmix™ Analysis Settings (cont.)*



Allele	D3S1358	vWA	D16S539	CSF1PO	TPOX	Yindel	D8S1179	D21S11	D18S51	DYS391	D2S441	D19S433	TH01	FGA	D22S1045	D5S818	D13S317	D7S820	SE33	D10S1248	D1S1656	D12S391	D2S1338
2.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0.00152	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0.00526	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0.009	0	0	0	0	0	0	0	0	0	0
5.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0.01274	0	0	0	0	0	0	0	0	0	0
6.2	0	0	0	0	0	0	0	0	0	0	0	0.00557	0	0	0	0	0	0	0	0	0	0	0
6.3	0	0	0	0	0	0	0	0	0	0	0	0	0.00152	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0.022641	0	0	0	0	0	0	0	0	0.01648	0	0	0.019387	0	0	0	0	0	0	0
7.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0.024804	0	0	0.029878	0	0	0	0	0	0.01238	0.02022	0	0	0.022402	0	0	0	0	0.02358	0
8.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0.009	0	0	0	0	0	0	0	0	0
9	0	0	0	0.030246	0	0	0.037661	0	0	0	0	0	0.01238	0.02396	0	0	0.030966	0	0	0	0	0.03176	0
9.3	0	0	0	0	0	0	0	0	0	0	0	0	0.01274	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0.036839	0	0	0.042471	0	0	0	0.030184	0.026	0.0277	0	0	0.043199	0	0	0	0	0	0.03585	0
10.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10.3	0	0	0	0	0	0	0	0	0	0	0	0	0.01274	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0.044241	0	0	0.049957	0	0	0	0.049777	0.03281	0.03144	0	0	0.050336	0	0	0	0	0	0.04812	0
11.1	0	0	0	0	0	0	0	0	0	0	0	0.026	0	0	0	0	0	0	0	0	0	0	0
11.2	0	0	0	0	0	0	0	0	0	0	0	0.03962	0	0	0	0	0	0	0	0	0	0	0
11.3	0	0	0	0	0	0	0	0	0	0	0.02136	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0.055077	0	0	0.054554	0	0	0	0.059071	0.03962	0.03518	0	0	0.057052	0	0	0.040696	0	0	0.05221	0
12.1	0	0	0	0	0	0	0	0	0	0	0	0.00557	0	0	0	0	0	0	0	0	0	0	0
12.2	0	0	0	0	0	0	0	0	0	0	0	0.04643	0	0	0	0	0	0	0	0	0	0	0
12.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0.063861	0	0	0.049493	0	0	0	0.037341	0.04643	0	0	0	0.065143	0	0	0.041997	0	0.0563	0	0
13.2	0	0	0	0	0	0	0	0	0	0	0	0.05324	0	0	0	0	0	0	0	0	0	0	0
13.3	0	0	0	0	0	0	0	0	0	0	0	0	0.02022	0	0	0	0	0	0	0	0.04812	0	0
14	0	0.035316	0	0.066012	0	0	0.059221	0	0	0	0.039798	0.05324	0	0	0	0	0	0	0.05354	0	0.06857	0	0
14.2	0	0	0	0	0	0	0	0	0	0	0	0.06005	0	0	0	0	0	0	0	0	0	0	0
14.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.03176	0	0
15	0	0.046947	0	0	0	0	0.058074	0	0	0	0.04247	0.06005	0	0	0	0	0	0	0.052187	0	0.07266	0	0
15.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15.2	0	0	0	0	0	0	0	0	0	0	0	0.06686	0	0	0	0	0	0	0	0	0	0	0
15.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.04403	0	0
16	0	0.051235	0	0	0	0	0.068086	0	0	0	0	0.06686	0	0	0	0	0	0	0.064185	0	0.08539	0	0
16.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16.2	0	0	0	0	0	0	0	0	0	0	0	0.07367	0	0	0	0	0	0	0	0	0	0	0
16.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.04812	0	0
17	0	0.062621	0	0	0	0	0.076603	0	0	0	0	0.07367	0	0	0	0	0	0	0.066869	0	0.08902	0	0
17.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17.2	0	0	0	0	0	0	0	0	0	0	0	0.077075	0	0	0	0	0	0	0	0	0	0	0
17.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0563	0	0
18	0	0.069018	0	0	0	0	0	0	0	0	0	0.08048	0	0.033941	0	0	0	0	0.073427	0	0.0972	0	0
18.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18.2	0	0	0	0	0	0	0	0	0	0	0	0.08729	0	0	0	0	0	0	0	0	0	0	0
18.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.06448	0	0
18.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0.074957	0	0	0	0	0	0	0	0	0	0	0	0.038142	0	0	0	0	0.08236	0	0	0	0
19.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19.2	0	0	0	0	0	0	0	0	0	0	0	0.0941	0	0	0	0	0	0	0	0	0	0	0
19.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.07266	0	0
19.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0.081606	0	0	0	0	0	0	0	0	0	0	0	0.045166	0	0	0	0	0.084203	0	0	0	0
20.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.050513	0	0	0	0
20.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0.051062	0	0	0	0	0.082513	0	0	0	0
21.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.053485	0	0	0	0
21.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0.056664	0	0	0	0	0	0	0	0	0
22.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0.056597	0	0	0	0	0.052382	0	0	0	0
22.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0.065492	0	0	0	0	0	0	0	0	0
23.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.060935	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0.068528	0	0	0	0	0	0	0	0	0
24.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.067043	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0.076072	0	0	0	0	0	0	0	0	0
25.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.070838	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0.081595	0	0	0	0	0	0	0	0	0
26.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.072745	0	0	0	0
27	0	0	0	0	0	0	0	0.046309	0	0	0	0	0	0.078408	0	0	0	0	0	0	0	0	0
27.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.079972	0	0	0	0
28	0	0	0	0	0	0	0	0.052354	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.092721	0	0	0	0
28.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0.060857	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.095201	0	0	0	0
29.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0.066497	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30.2	0	0	0	0	0	0	0	0.050642	0	0	0	0	0	0	0	0	0	0	0.08911	0	0	0	0
30.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0.067552	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31.2	0	0	0	0	0	0	0	0.049855	0	0	0	0	0	0	0	0	0	0	0.099899	0	0	0	0
32	0	0	0	0	0	0	0	0.084773	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32.2	0	0	0	0	0	0	0	0.070175	0	0	0	0	0	0	0	0	0	0	0.108434	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33.2	0	0	0	0	0	0	0	0.076163	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Figure 5 – GlobalFiler™ stutter values included in the Stutter Exceptions File where longest uninterrupted stretch (LUS) information is available

Appendix B: *GlobalFiler™ STRmix™ Analysis Settings (cont.)*

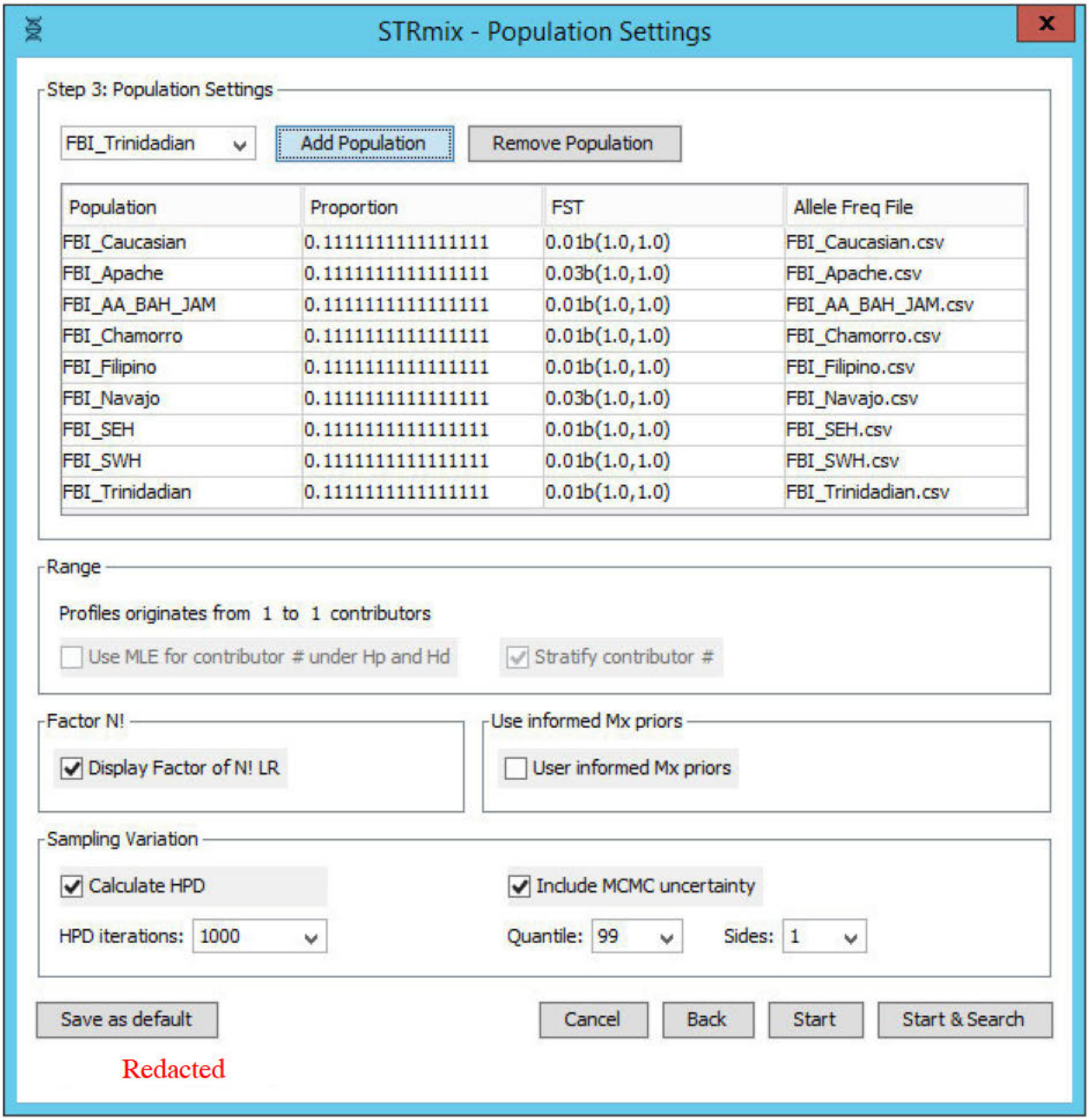


Figure 6 – STRmix™ V2.4.05 Population options and parameters

Appendix C: *Glossary*

Adenylation – also referred to as non-template-dependent nucleotide addition. *Taq* DNA polymerase is known to add an additional nucleotide (typically "A") to the 3' ends of double-stranded PCR products in a non-template-dependent manner. This phenomenon results in the generation of the "plus-A" fragment which is the allele (N) produced by amplification. When an additional nucleotide is not added to a fragment, it results in a "minus-A" fragment, an artifact of the PCR process.

Allelic Ladder – a mixture of common alleles which is run separate from any sample(s) or control(s), and is used by the software as a reference for designating alleles. Each ladder is a kit reagent that consists of amplified allelic fragments of known size and repeat content. The ladder does not contain all possible alleles that could be detected at an individual STR locus (e.g., off-ladder alleles).

Alternate Reference Sample – a specimen of established origin that may be used as a reference sample (e.g., a DNA typing result obtained from blood on the clothing of a victim known to have been bleeding or an item of personal effect).

Analytical Threshold (AT) – the minimum peak height that confidently ascribes a true amplicon peak and the height below which confidence is too low to reliably assign a peak as an allele. The AT is higher than the limit of detection of the system to increase the confidence that any given peak at or above this threshold is a PCR product.

Concordance – agreement in allele calls/DNA types at shared loci between different amplifications of the same sample. Agreement does not require that the allele calls be identical but rather that they were amplified from a common source. Determination of concordance between different amplifications of a sample must take into account kit differences and differences in the quantity of the DNA amplified. Concordance can be also determined for samples creating a composite DNA profile (e.g., different extractions of the same stain or the same bone); however, these determinations must additionally take into account differences resulting from sampling and extraction (e.g., efficiency of the differential).

Conditional Reference Sample (i.e., assumed contributor) – the reference sample from an individual that corresponds to an evidence item taken from an anatomical location (e.g., vaginal swab, oral swab, fingernail clippings) or item of direct physical contact (e.g., under shorts, panties, bra) which is expected to yield DNA from the individual from whom the specimen was taken.

Contamination – the unintentional introduction of foreign DNA into a sample. Can be categorized as **random** or **systemic**.

Daughter Plate – the plate containing samples that have been prepared for CE. Prepped samples usually consist of a mixture of formamide, size standard, and amplified DNA.

Differential Extraction – a type of extraction that procedurally subdivides the sample into male and female fractions. The female/non-sperm/F fraction is enriched for DNA of non-sperm origin such as epithelial cells and white blood cells. The male/sperm/M fraction is enriched for DNA of sperm cell origin.

Disassociated Primer Dye – a dye that has become detached from its primer resulting in non-specific, reproducible peaks. These peaks may be specific to a given lot of amplification kits.

Discordance – Non-agreement in allele calls/DNA types at shared loci between different amplifications of purportedly the same sample that cannot be explained by differences in the amplification kit used and/or differences in the quality or quantity of the DNA amplified. Discordance indicates that the results were from different sources. Discordance may be also determined between different extractions of the same stain or the same bone.

Distinguishable Mixture – a mixture of DNA in which donors contributed different amounts of biological material to the sample, resulting in the ability to attribute alleles to an individual donor(s).

Dropout – the failure to detect an allele(s) in a sample usually due to stochastic amplification of low levels of DNA.

Extraction Control – contains all of the chemical solutions used in the analysis process except any DNA containing sample and is processed through the same extraction, quantitation, amplification, and electrophoretic typing procedures as the evidentiary specimens. An extraction control monitors aspects of the analytical processes for the introduction of adventitious DNA.

F_{ST}–the chance that two alleles from two different people are identical by descent.

GeneScan®-500 Internal Size Standard (GS-500) – consists of DNA fragments of known sizes (35, 50, 75, 100, 139,150,160, 200, 250, 300, 340, 350, 400, 450, 490, and 500 bp) that are labeled with an orange dye (GS-500 LIZ), and are combined with an aliquot of each amplified sample during daughter plate preparation prior to electrophoresis. This standard is used to size peaks that are detected by the CE instrument that are differentiated with blue, green, yellow, and red fluorescent dyes. Due to the temperature sensitivity of the 250 bp fragment's sequence-based conformation, this fragment is usually not used for sizing purposes.

GeneScan®-600 Internal Size Standard Version 2 (GS-600v2) – consists of DNA fragments of known sizes (20, 40, 60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440, 460, 480, 500, 514, 520, 540, 560, 580 and 600bp) that are labeled with an orange dye (GS-600 LIZ), and are combined with an aliquot of each amplified sample during daughter plate preparation prior to electrophoresis. This standard is used to size peaks that are detected by the CE instrument that are differentiated with blue, green, yellow, and red and purple fluorescence dye. Due to the temperature sensitivity of the 250 bp fragment's sequence-based conformation, this fragment is usually not used for sizing purposes.

Indistinguishable Mixture – a typing result from a sample for which alleles cannot be attributed to individual donors, which occurs when similar amounts of biological material are contributed to the specimen by multiple donors.

Local Southern Method – used for size calling samples amplified with the GlobalFiler™ Kit. This method uses the four fragments closest in size to the unknown fragment to determine a best fit line. This best fit line is an average of two such curves created by using three standard points each (the first makes use of the two points below and one point above the unknown fragment and the other one point

below and two points above).In this way, only the region of the size standard near the fragment(s) of unknown length is analyzed.

Major Contributor – the component of a distinguishable mixture who donated the preponderance of DNA. When using STRmix™, a major contributor is the contributor with the highest mixture proportion of DNA, which is at least double that of the next contributor's DNA proportion.

MCMC – Markov Chain Monte Carlo; an algorithm based on standard mathematical principles to assign weights to genotype combinations.

Microvariant – an allele that contains an incomplete repeat unit.

Minor Contributor – the component of a mixture who donated a lesser amount of DNA in relation to the major contributor.

Minus A – a product of the amplification process which results in a peak that is one base pair shorter than the allelic fragment due to the failure to add a nucleotide to the 3' end. The height of the –A peak is generally <15% of the allelic peak. It is more likely to be observed when excessive template DNA amounts are used in the PCR and/or when an inhibitor of *Taq* DNA Polymerase is present in the sample.

Negative Amplification Control – contains all of the chemical components required for the amplification of DNA, with the exception of any DNA sample or extraction control. It is processed through the same amplification and electrophoretic typing procedures as any sample(s), and is used to monitor aspects of the analytical processes for the introduction of adventitious DNA that may have occurred during or after amplification.

Off-ladder Allele (OL) – DNA fragments of genetic origin that fail to size within a defined allele category (e.g., a bin or virtual bin).

Person of Interest (POI) – the person for which you are determining the weight of the evidence. The POI can be the subject, the victim, or any individual for whom you have a reference sample.

Positive Amplification Control – contains all of the chemical components required for the amplification of DNA including a known DNA source (e.g., 9947A or 007), and serves as a general qualitative indicator of amplification, as well as a confirmation that the software has functioned accurately in assigning alleles.

Proposition – describes the two hypotheses being compared. One hypothesis is that an individual is included as a contributor to the evidence along with additional unknown, unrelated individual(s), as needed. The other hypothesis is that the evidence originates from unknown, unrelated individual(s). In some instances, an assumed contributor may be included in both hypotheses.

Pull-up – a low intensity peak that derives from excessive fluorescence intensity of another peak and generally observed in the color(s) spectrally adjacent to the high intensity peak.

Raised Baseline – appears in an electropherogram as a non-specific elevation of the horizontal axis between one or more peaks. This elevation can result from excessive template DNA or be instrument related (e.g., a misaligned capillary).

Random Contamination – incidents of adventitious DNA that occur in a manner that suggests that the source of extraneous DNA was introduced into a sample(s) or control(s) and was not a part of a material and/or a reagent common to all of the samples processed as a batch. Extremely low level contamination, such as a result at the amelogenin locus with no results at any STR loci, may also be considered random. Additionally, if one of multiple reagent blanks within a batch was contaminated, the conclusion of random contamination may be supported by demonstrating that another reagent blank from the batch was not contaminated. (e.g., by an equally or more sensitive amplification, if no signal was indicated during quantitation).

Spike – non-specific, non-reproducible peak(s) that may result during electrophoresis from electrical fluctuations in the power source, from the interference of urea crystals, bubbles, or inherently fluorescent materials such as detergent in the capillary.

Stochastic Threshold – an empirically determined parameter that specifies the minimum peak height that all allelic peaks at a given locus must display to be confident that no genetic components of a sample failed to be detected due to differential amplification (i.e., stochastic loss due to low template mass, degraded template DNA, and/or PCR inhibition). This parameter is established for each amplification kit.

Stutter – phenomenon inherent to the PCR amplification of repetitive DNA sequences, and often is presented as a minor PCR product peak one repeat unit smaller (e.g., N-4) than the source allelic peak (N).Stutter peaks originate *in vitro* (i.e., the PCR reaction) from an allele that exists *in vivo* (i.e., the template DNA) and do not constitute an individual's genotype. The size and intensity of stutter peaks are generally predictable based on the alleles that are detected in a specimen.

STRmix™ – a software system that applies a fully continuous probabilistic genotyping approach to DNA profile interpretation. It standardizes the analysis of profiles within a laboratory by using estimates of variance of electropherograms derived from the laboratory's own DNA profiling data.

Systemic Contamination – incidents of adventitious DNA that occur in a manner that suggests that the source of the introduced DNA was a material and/or a reagent common to some or all of the samples processed as a batch. It is noted that systemic contamination may not affect all samples equally and may be present in some but not all of the samples in the batch. Additionally, contamination may be systemic to an extraction batch without affecting the other extraction batches in an amplification batch.

Trace Contributor – a contributor whose alleles exhibit low amplitude, as well as possible allele dropout and/or imbalance of heterozygous alleles due to stochastic amplification.

Tri-allele – a rare anomaly resulting in three alleles at a locus in a single individual.

Virtual Bin – an allele sizing category designated by the software that has no corresponding allele physically present in the ladder.

Appendix D: *Allele frequency data for Minnesota Native American Population*

GlobalFiler™ (Life Technologies, Inc.) data (i.e., fsa files) for the Minnesota Native American population samples were generated by the Minnesota Bureau of Criminal Apprehension and provided to the FBI Laboratory DNA Support Unit (DSU). Genotyping was performed using GeneMapper® ID-X software version 1.4 (Life Technologies, Inc.). Microsoft Excel was used to calculate allele frequencies. Arlequin version 3.5.2.2 (Excoffier 2010) was used to test the hypothesis that none of the loci departed from Hardy-Weinberg equilibrium ($p>0.05$). Three of 21 markers yielded a p value <0.05; however, after applying the Bonferroni correction, the affected loci were found to also be in equilibrium. This dataset is suitable in terms of both size and quality for the purposes of estimating DNA profile probabilities. It is noted that N is equal to the number of alleles typed.

Appendix D: *Allele frequency data for Minnesota Native American Population (cont.)*

Minnesota Native American Population Globalfiler Expanded STR Loci Allele Frequencies

Allele	D3S1358	vWA	D16S539	CSF1PO	TPOX	Y indel	D8S1179	D21S11	D18S51	DYS391	D2S441	D19S433	TH01	FGA	D22S1045	D5S818	D13S317	D7S820	SE33	D10S1248	D1S1656	D12S391	D2S1338	Allele
2						1.000000																		2
6													0.195946											6
7					0.003378								0.442568			0.165541								7
8			0.006757	0.006757	0.405405		0.006757						0.037162				0.057432	0.128378						8
9			0.141892	0.087838	0.043919		0.003378			0.213115	0.016892		0.077703			0.057432	0.179054	0.081081						9
9.3													0.243243											9.3
10			0.162162	0.320946	0.030405		0.050676		0.010135	0.426230	0.277027		0.003378			0.047297	0.128378	0.209459						10
11	0.003378		0.290541	0.202703	0.388514		0.027027			0.344262	0.442568	0.003378			0.094595	0.391892	0.226351	0.266892		0.027027	0.034014			11
11.3											0.020270													11.3
12		0.003401	0.263514	0.327703	0.128378		0.125000		0.131757	0.016393	0.037162	0.033784			0.003378	0.236486	0.253378	0.277027	0.010135	0.013514	0.061224			12
13		0.003401	0.108108	0.043919			0.351351		0.091216		0.023649	0.168919				0.097973	0.067568	0.037162		0.287162	0.098639			13
13.2											0.158784													13.2
14	0.064189	0.040816	0.027027	0.010135			0.310811		0.293919		0.165541	0.239865			0.013514	0.003378	0.084459		0.010135	0.334459	0.068027			14
14.2												0.040541												14.2
14.3																				0.003401				14.3
15	0.418919	0.074830					0.101351		0.128378		0.016892	0.141892			0.405405		0.003378		0.016892	0.212838	0.142857	0.010135		15
15.2												0.108108												15.2
15.3																				0.030612				15.3
16	0.310811	0.340136					0.016892		0.111486			0.037162			0.429054				0.033784	0.108108	0.221088	0.010135	0.013514	16
16.2												0.023649												16.2
16.3																					0.047619			16.3
17	0.108108	0.323129					0.006757		0.125000			0.006757			0.030405				0.037162	0.013514	0.054422	0.040541	0.104730	17
17.2												0.027027												17.2
17.3																					0.153061	0.010135		17.3
18	0.084459	0.156463							0.030405				0.010135	0.016892					0.091216	0.003378	0.273649	0.060811		18
18.2												0.010135				0.006757							18.2	
18.3																			0.003378		0.074830	0.003378		18.3
19	0.010135	0.040816							0.040541				0.101351	0.006757					0.074324		0.216216	0.307432		19
19.2																					0.027027			19.2
19.3																					0.006803			19.3
20		0.013605							0.020270					0.128378					0.057432		0.135135	0.091216		20
20.2																			0.003378					20.2
21		0.003401							0.006757					0.128378					0.040541		0.094595	0.027027		21
21.2																			0.013514					21.2
22									0.006757					0.108108					0.016892		0.067568	0.145270		22
22.2														0.003378					0.023649					22.2
23									0.003378					0.148649					0.020270		0.091216	0.128378		23
23.2																			0.006757					23.2
24														0.185811							0.016892	0.087838		24
24.2																			0.013514					24.2
25														0.128378							0.003378	0.030405		25
25.2																			0.027027					25.2
26														0.043919								0.003378		26
26.2														0.003378					0.070946					26.2
27								0.006757						0.006757										27
27.2																			0.064189					27.2
28								0.064189						0.003378										28
28.2																			0.097973					28.2
29								0.185811																29
29.2																			0.097973					29.2
30								0.327703																30
30.2								0.023649											0.101351					30.2
31								0.067568																31
31.2								0.185811											0.043919					31.2
32								0.003378																32
32.2								0.081081											0.006757					32.2
33								0.003378											0.010135					33
33.2								0.040541																33.2
34.2								0.010135																34.2
Allele	D3S1358	vWA	D16S539	CSF1PO	TPOX	Y indel	D8S1179	D21S11	D18S51	DYS391	D2S441	D19S433	TH01	FGA	D22S1045	D5S818	D13S317	D7S820	SE33	D10S1248	D1S1656	D12S391	D2S1338	Allele
N	296	294	296	296	296	61	296	296	296	122	296	296	296	296	296	296	296	296	296	296	294	296	296	

Appendix E: *Interpretation of Identifiler® Plus data*

When making conclusions, the reevaluation of any of the allele calls or genotype calls, removal of alleles (or entire loci) from statistical estimates, or a change in the assumptions is considered reinterpretation. The DNA examiner must be previously qualified in the interpretation of data from the legacy amplification kit and platform instrument model and authorized to perform reinterpretation of legacy data.

1. GMIDX settings

The GMIDX software settings are represented in the procedure for interpretation of results from the Identifiler® Plus amplification kit (i.e., DNA 229) and/or the procedures for interpretation of legacy DNA data (i.e., DNA 230).

2. Non-Specific Peaks

Non-specific peaks are defined as peaks of unknown origin. Those known to be associated with specific commercial products are noted below.

a. Non-specific peaks of multiple colors have been observed in some lots of the GS-500. Because the GS-500 is added to each sample in preparation for electrophoresis, these non-specific peaks that originate from the GS-500 can be seen in any sample.

b. Excessive non-specific peaks are defined as peaks of unknown origin at two or more loci that result in stretches of non-specific, low amplitude data. Samples exhibiting excessive non-specific peaks may have been be re-injected for reduced time(s) to facilitate interpretation.

3. Non-Human Peaks

The amplification of non-human DNA may exhibit characteristics such as:

a. A peak at ~98 bp (before amelogenin) and/or randomly at other loci.

b. A peak at D21S11 called a “28.2”.

c. Alleles absent at the majority of the locations but not consistent with a degraded profile or low level human DNA sample

Non-human DNA typing results should not be used for comparisons.

Appendix E: *Interpretation of Identifiler® Plus data (cont.)*

4. Stutter

The Identifiler® Plus (27 or 28 cycles) stutter percentage guidelines provided in Table 4 are estimates (Average + 3 SD) of the maximum expected relative negative stutter, or N-4, values Positive stutter, or N+4, can also occur, and is typically less than 5% for all loci.

Locus	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539
% Stutter	11	12	10	11	13	6	12	11

Locus	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
% Stutter	13	13	13	7	15	11	13

Table 1 – Maximum Expected Stutter Percentage Guidelines

5. Application of Peak Height Thresholds to Allelic Peaks

5.1 The analytical threshold (AT) is 50 RFU.

5.2 The stochastic thresholds (ST) are:

- 200 RFU for the Identifiler® Plus (27 cycles) Amplification Kit
- 300 RFU for the Identifiler® Plus (28 cycles) Amplification Kit

6. Peak Height Ratio

The values provided in Table 2 are estimates for the minimum expected PHR percentages for the AmpF*l*STR® Identifiler® Plus (27 cycles) Amplification Kit and are based on the average PHRs observed in the validation studies. The PHR guidelines are only applicable to allelic peaks that meet or exceed the ST.

Peak Height	All Identifiler® Plus STR Loci (27 cycles)
200-499 RFU	50%
500-999 RFU	60%
1000 RFU and above	70%

Table 2 – Minimum Expected Heterozygous Peak Height Ratio Guidelines (3130xl Data)

Appendix E: *Interpretation of Identifiler® Plus data (cont.)*

7. DNA Typing Results Subjected to STRmix™ Interpretation

7.1 STRmix™ analysis may be conducted on evidentiary typing results amplified using the Identifiler® Plus Kit with 27 cycles and separated on the Applied Biosystems 3130XL for STRmix™ analysis. Reference profiles are not constrained by these requirements for STRmix™ analysis.

7.2 Typing results assumed to originate from 1 to 4 contributors may be interpreted using STRmix™. In addition, if conditioned on at least one contributor, a 5-person mixture may be interpreted using STRmix™. Five-person mixtures without an assumed contributor, as well as mixtures of DNA from more than 5 contributors, will not be interpreted using STRmix™, and will be reported as unsuitable for comparisons.

8. STRmix™ Settings and Usage

8.1 STRmix™ software settings for versions 2.3.06 and 2.4.05, including FBI specific parameters from internal validation studies, are depicted in Appendix F.

8.2 The latest version of STRmix™ validated for Identifiler® Plus Kit will generally be used for analysis.

8.3 If STRmix™ was previously run and statistics reported for a person of interest (POI), the LR from previous (LRFP) function will be used for analysis of additional POIs. The LRFP function is restricted to the version of STRmix™ used for the original deconvolution. If the original deconvolution is not available, it will be recreated in STRmix™ by setting the seed in the version used for the original deconvolution.

8.4 If STRmix™ was previously run solely for the purposes of a CODIS deconvolution, the LRFP function may be used for analysis of POIs submitted at a later time. Alternatively, a new deconvolution using the latest version of STRmix™ may be performed.

9. Unsuitable Results

Five-person mixtures without an assumed contributor, as well as mixtures of DNA from more than 5 contributors, will be reported as unsuitable for analysis.

Appendix F: *Identifiler® Plus STRmix™ Analysis Settings*

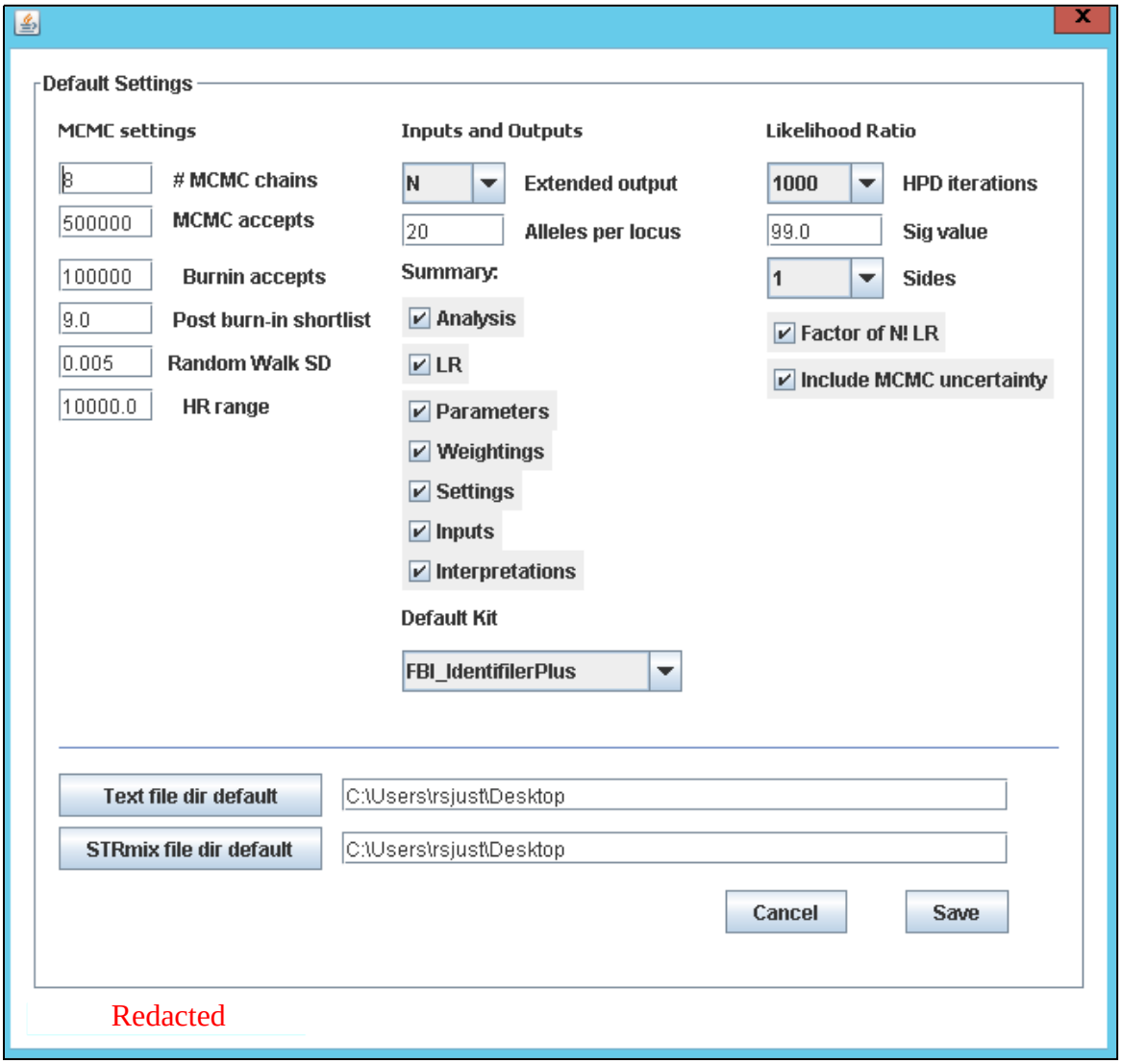


Figure 1 – STRmix™ V2.3.06 default settings
(refer to Appendix B Figure 1 for STRmix™ V2.4.05 default settings)

Appendix F: *Identifiler® Plus STRmix™ Analysis Settings (cont.)*

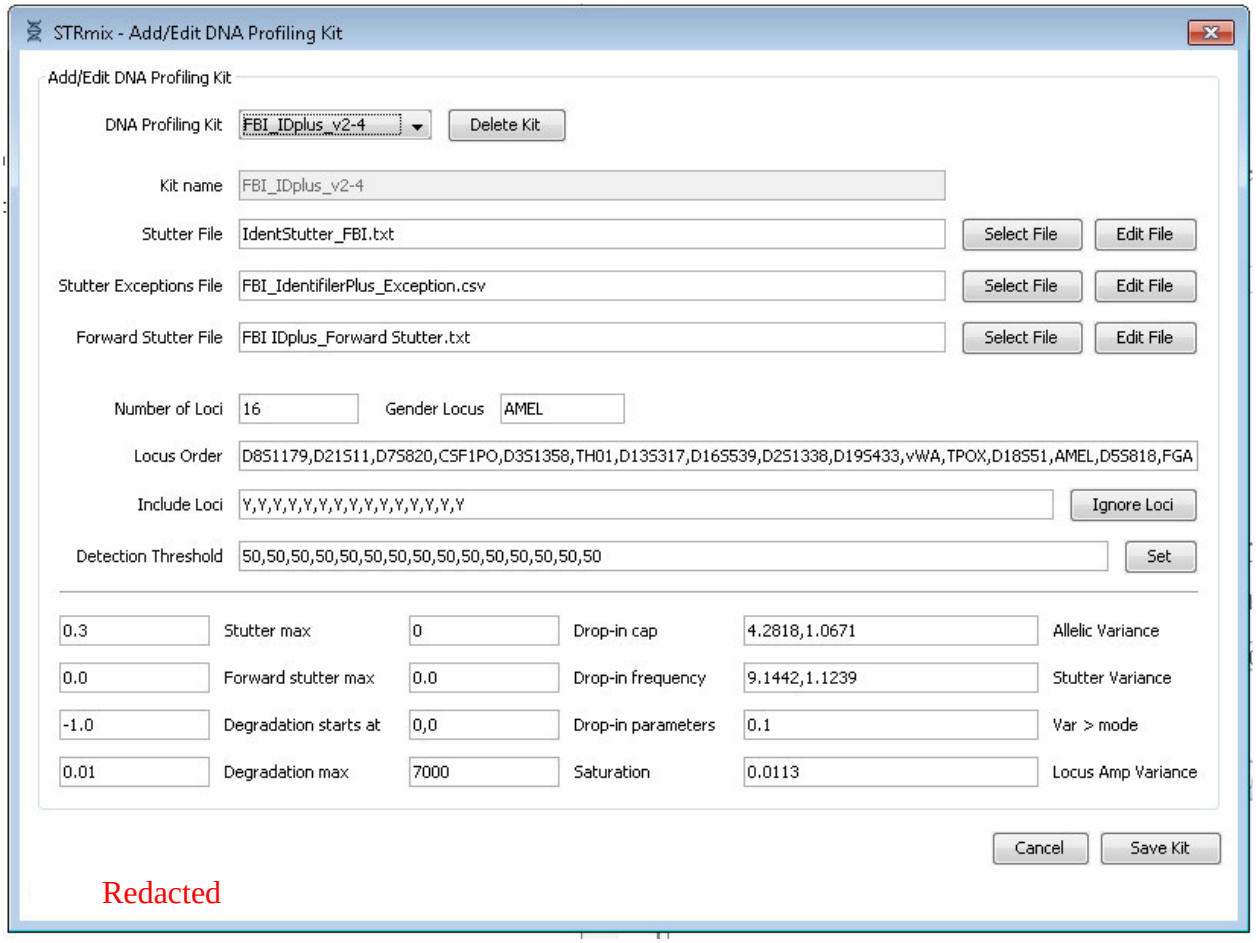


Figure 2 – STRmix™ V2.4.05 Identifiler® Plus kit settings

Appendix F: *Identifiler® Plus STRmix™ Analysis Settings (cont.)*

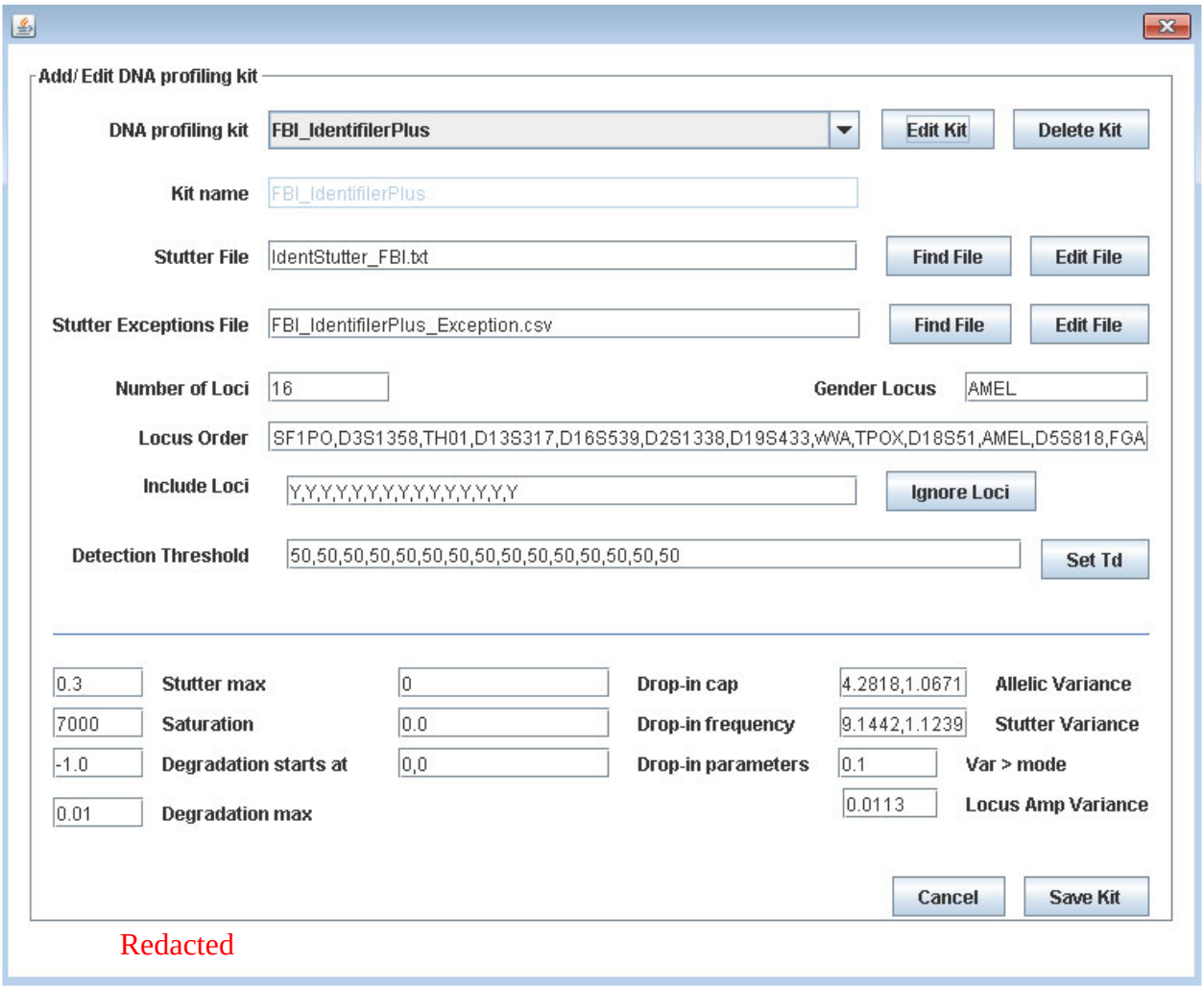


Figure 3 - STRmix™ V2.3.06 Identifiler® Plus kit settings

Appendix F: *Identifiler® Plus STRmix™ Analysis Settings (cont.)*

```
Locus,Intercept,Slope
1,0.0087,0.00378
2,-0.0725,0.0046
3,-0.0446,0.00868
4,-0.0565,0.00971
5,-0.0446,0.00773
6,-0.0102,0.00409
7,-0.046,0.00829
8,-0.0529,0.00948
9,-0.00363,0.00369
10,-0.0672,0.00987
11,-0.0595,0.00752
12,-0.0243,0.00587
13,-0.0155,0.00594
14,-0.0442,0.00884
15,-0.0357,0.00468
```

Figure 4 – Parameters used in determination of allele-specific stutter ratios at Identifiler® Plus loci

Appendix F: *Identifiler® Plus STRmix™ Analysis Settings (cont.)*

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
5	0	0	0	0	0	0.0072	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0.01258	0	0	0	0	0	0	0	0	0
7	0.0289	0	0	0	0	0.01796	0	0	0	0	0.05207	0	0	0	0
8	0.0351	0	0	0	0	0.02334	0	0	0	0	0	0	0	0	0
8.3	0	0	0	0	0	0.0072	0	0	0	0	0	0	0	0	0
9	0.0413	0	0	0	0	0.02872	0	0	0	0	0	0	0	0	0
9.3	0	0	0	0	0	0.01258	0	0	0	0	0	0	0	0	0
10	0.0475	0	0	0	0	0.0541	0	0	0	0.03318	0	0	0	0	0
10.3	0	0	0	0	0	0.01796	0	0	0	0	0	0	0	0	0
11	0.0537	0	0	0	0.03143	0.03948	0	0	0	0.04209	0.05207	0	0	0	0
11.3	0	0	0	0	0	0.02334	0	0	0	0	0	0	0	0	0
12	0.0599	0	0	0	0.04012	0	0	0	0	0.051	0	0	0	0	0
12.1	0	0	0	0	0	0	0	0	0	0.00645	0	0	0	0	0
12.2	0	0	0	0	0	0	0	0	0	0.05991	0	0	0	0	0
13	0.0537	0	0	0	0.04881	0	0	0	0	0.05991	0.05207	0	0	0	0
13.2	0	0	0	0	0	0	0	0	0	0.06882	0	0	0	0	0
14	0.0599	0	0	0	0.06619	0	0	0	0	0.06882	0.035675	0	0	0	0
14.2	0	0	0	0	0	0	0	0	0	0.07773	0	0	0	0	0
15	0.0661	0	0	0	0.070535	0	0	0	0.05184	0.07773	0.057305	0	0	0	0
15.2	0	0	0	0	0	0	0	0	0	0.08664	0	0	0	0	0
16	0.0661	0	0	0	0.079225	0	0	0	0.05834	0.08664	0.064515	0	0	0	0
16.2	0	0	0	0	0	0	0	0	0	0.09555	0	0	0	0	0
17	0.0661	0	0	0	0.087915	0	0	0	0.06484	0.09555	0.06812	0	0	0	0.04038
17.2	0	0	0	0	0	0	0	0	0	0.10446	0	0	0	0	0
18	0.0785	0	0	0	0.09226	0	0	0	0.07134	0.10446	0.071725	0	0	0	0.04572
18.2	0	0	0	0	0	0	0	0	0	0.11337	0	0	0	0	0
19	0	0	0	0	0.10095	0	0	0	0.06809	0	0.08254	0	0	0	0.05106
19.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0564
20	0	0	0	0	0.10964	0	0	0	0.06484	0	0.08975	0	0	0	0.0564
20.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.06174
21	0	0	0	0	0	0	0	0	0.06484	0	0.09696	0	0	0	0.06174
21.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.06708
21.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0297
22	0	0	0	0	0	0	0	0	0.07134	0	0.10417	0	0	0	0.06708
22.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.07242
23	0	0	0	0	0	0	0	0	0.07784	0	0	0	0	0	0.07242
23.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.07776
24	0	0	0	0	0	0	0	0	0.08434	0	0	0	0	0	0.07776
24.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.07776
24.2	0	0.0443	0	0	0	0	0	0	0	0	0	0	0	0	0.0831
25	0	0.0592	0	0	0	0	0	0	0.09084	0	0	0	0	0	0.0831
25.2	0	0.0592	0	0	0	0	0	0	0	0	0	0	0	0	0.08844
26	0	0.0443	0	0	0	0	0	0	0.09734	0	0	0	0	0	0.08844
26.2	0	0.06665	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0.049217	0	0	0	0	0	0	0.10384	0	0	0	0	0	0.07776
28	0	0.055475	0	0	0	0	0	0	0.11034	0	0	0	0	0	0.09912
28.2	0	0.0592	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0.062925	0	0	0	0	0	0	0	0	0	0	0	0	0.07242
29.2	0	0.0592	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0.06665	0	0	0	0	0	0	0	0	0	0	0	0	0
30.2	0	0.0592	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0.070375	0	0	0	0	0	0	0	0	0	0	0	0	0
31.2	0	0.06665	0	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0.08155	0	0	0	0	0	0	0	0	0	0	0	0	0
32.2	0	0.079017	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0.089	0	0	0	0	0	0	0	0	0	0	0	0	0
33.2	0	0.079017	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0.08155	0	0	0	0	0	0	0	0	0	0	0	0	0
34.2	0	0.089	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0.06665	0	0	0	0	0	0	0	0	0	0	0	0	0
35.2	0	0.09645	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0.0741	0	0	0	0	0	0	0	0	0	0	0	0	0
36.2	0	0.1039	0	0	0	0	0	0	0	0	0	0	0	0	0
37.2	0	0.089	0	0	0	0	0	0	0	0	0	0	0	0	0
38.2	0	0.08155	0	0	0	0	0	0	0	0	0	0	0	0	0
39.2	0	0.08155	0	0	0	0	0	0	0	0	0	0	0	0	0

Figure 5 – Identifiler® Plus stutter values included in the Stutter Exceptions File where LUS information is available

Appendix F: *Identifiler® Plus STRmix™ Analysis Settings (cont.)*

Figure 6 – STRmix™ V2.3.06 Population options and parameters for calculation of the LR (refer to Appendix B Figure 6 for STRmix™ V2.4.05 options and parameters)